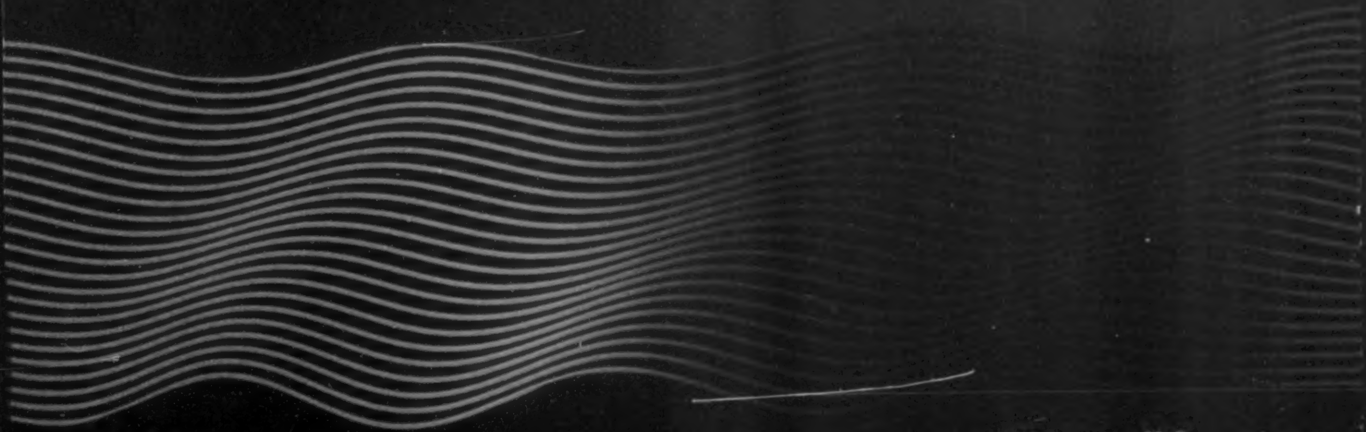
Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



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Distribution of *Nitzschia pungens* in Coastal Waters of British Columbia

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Concern about the potential for contamination of Pacific coast molluscan shellfish by domoic acid prompted us to review the distribution of *Nitzschia pungens* in coastal waters of British Columbia. From 1980 to 1988, *N. pungens* occurred throughout waters of the continental shelf, most frequently as a minor component of the large diatom aggregations observed off southwest Vancouver Island during July and August. The species was less common in the Strait of Georgia and north of Vancouver Island, but interannual variability in distribution and abundance was considerable. Maximum concentrations recorded were 10^6 cells·L^{-1} in Hecate Strait in July 1983 and 5×10^5 cells·L^{-1} off southwest Vancouver Island in August 1986. Discrimination of presence or absence on the basis of existing environmental variables produced mixed results, but has potential. Scanning electron microscopy showed that both *N. pungens* f. *pungens* and *N. pungens* f. *multiseries* (the latter being implicated in the contamination of shellfish in Atlantic Canada) occur in British Columbia. It remains to be shown whether *N. pungens* produces domoic acid in Pacific coast waters and whether the high abundances observed over the continental shelf also occur near shore.

En raison des craintes que suscite la possibilité de la contamination à l'acide domoïque des mollusques de la côte du Pacifique, nous avons étudié la distribution de *Nitzschia pungens* dans les eaux de la côte de la Colombie-Britannique. De 1980 à 1988, *N. pungens* était présent partout dans les eaux de la plate-forme continentale, constituant le plus souvent l'un des composants mineurs des grands agrégats de diatomées observés au large du sud-ouest de l'île de Vancouver en juillet et en août. Il était moins répandu dans le détroit de Georgie et au nord de l'île de Vancouver, mais la variabilité interannuelle de la distribution et de l'abondance était considérable. On a mesuré concentrations maximales suivantes : 10^6 cellules·L^{-1} dans le détroit d'Hecate en juillet 1983 et 5×10^5 cellules·L^{-1} au large du sud-ouest de l'île de Vancouver en août 1986. La détermination de la présence de l'organisme d'après les variables du milieu a donné des résultats inégaux, mais la méthode a du potentiel. L'examen au microscope électronique à balayage a révélé que *N. pungens* f. *pungens* et *N. pungens* f. *multiseries* (forme mise en cause dans la contamination des mollusques de l'Atlantique canadien) sont tous deux présents en Colombie-Britannique. Il reste à déterminer si *N. pungens* produit de l'acide domoïque dans les eaux côtières du Pacifique et s'il est présent près des côtes en aussi grand nombre que dans la zone de la plate-forme continentale.

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In November 1987, cultured mussels harvested from Cardigan Bay, Prince Edward Island, were found to be contaminated with the toxin domoic acid. Investigation by Subba Rao et al. (1988) and Bates et al. (1989) indicated that the source of the contamination was a massive bloom of the diatom *Nitzschia pungens* Grunow f. *multiseries* Hasle (Hasle 1965). The event in Prince Edward Island was the first record of domoic acid as a shellfish contaminant. At least three deaths and more than 100 cases of illness were attributed to this event (Subba Rao et al. 1988; Perl et al. 1990). Shellfish toxicity caused by domoic acid has been named amnesic shellfish poisoning (ASP) (Bates et al. 1989) due to the nature of the neurological symptoms that are produced (Debonnel et al. 1989; Perl et al. 1990). Similar blooms to that in 1987 occurred in the same locations in 1988 and 1989 (Smith et al. 1989).

The molluscan shellfish industry in British Columbia is substantial. Commercial landings of wild stocks exceeded 9500 tonnes in 1988 (Department of Fisheries and Oceans (Canada) 1988), while cultivated production of oysters, the major producing species in the province, was about 3700 tonnes in 1986 (House of Commons (Canada) 1988). Concern about the potential for contamination by domoic acid prompted us to review the distribution of *N. pungens* in coastal waters of British Columbia. We have reviewed our records of this species based on phytoplankton samples taken from 1980 to 1988. These were collected in conjunction with studies on the control by oceanographic processes of the planktonic ecosystem over the continental shelf of British Columbia. Additional samples from the subarctic Pacific and coastal inlets were also collected. We have examined the distribution and abundance of *N. pungens* by geographic area and by time of year. In addition, we have compared the water properties characteristic of samples that contain the species with those that do not.

Methods

We obtained phytoplankton samples in Niskin bottles, usually attached to a rosette sampler equipped with a CTD, in situ fluorometer, and transmissometer (Denman et al. 1985) or, in some cases, attached to a hydro wire, or in surface buckets.

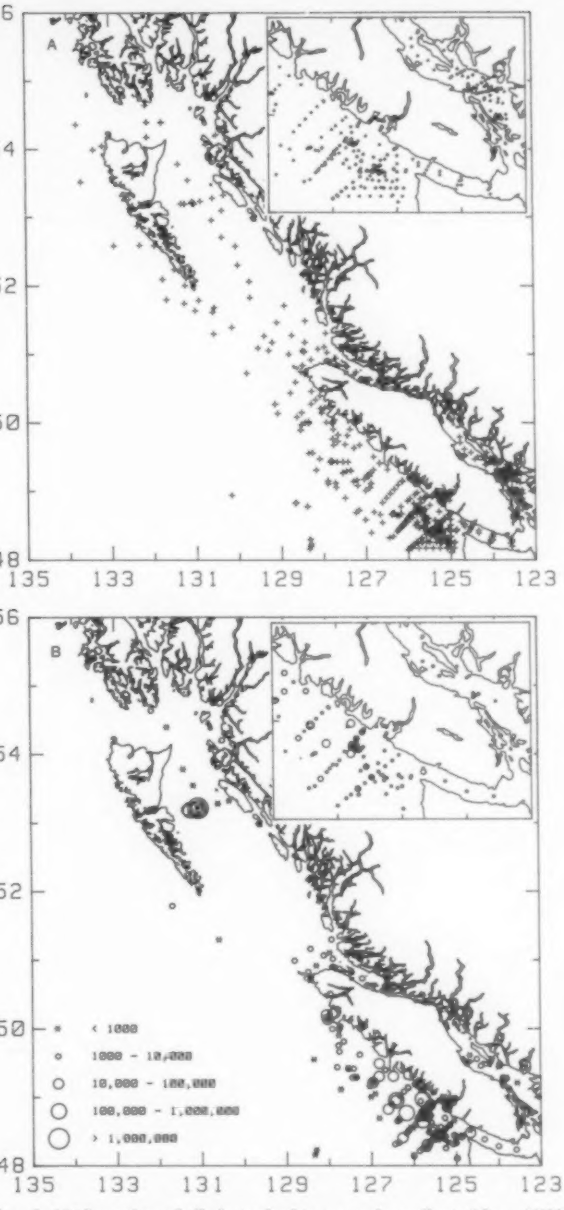

FIG. 1. (A) Location of all phytoplankton samples collected from 1980 to 1988. Inset: detail of southern Vancouver Island area. (B) Location of all samples containing *N. pungens*, with abundances (cells·L^{-1}) represented by circle diameters. Inset: as in Fig. 1A, with relative abundance indicated by circle diameters.

Samples were preserved with acidic Lugol's fixative (Throndsen 1978) and photoautotrophic taxa were identified and enumerated by inverted microscopy (Hasle 1978). Samples for use in scanning electron microscopy were prepared in a similar manner to the method employed by Bates et al. (1989) and examined in a Cambridge S-100 scanning electron microscope.

Data collected with the phytoplankton samples varied according to changing research objectives. Generally included were temperature, salinity, and chlorophyll. Concentrations of reactive nitrate plus nitrite, phosphate, and silicate, as well as radioactive carbon uptake rates were frequently determined. Methods employed are described in Denman et al. (1985).

TABLE 1. Number of samples with *N. pungens* present, by month, compared with the total, for all samples from 1980 to 1988. The percentage of samples with the species present is listed at the bottom.

	Apr.	May	June	July	Aug.	Sept.	Oct.	Total
N. pungens	1	47	26	67	81	2	0	224
Total samples	4	231	156	235	138	20	18	802
% of total	25	20	17	29	59	10	0	28

Our sampling has been concentrated off the southwest coast of Vancouver Island and in the Strait of Georgia, with less dense coverage in other coastal waters (Fig. 1A). Sampling has generally been conducted from large research vessels on the continental shelf and in adjacent oceanic waters. Few samples have been taken in coastal inlets or near shore. Exceptions include several samples taken in Alice Arm on the extreme north coast in August 1981, a few samples collected in Saanich Inlet on southeast Vancouver Island at various times, and an extensive set of samples from Barkley Sound on the west coast of Vancouver Island acquired in May 1988. Records are restricted to the months from April to October (Table 1), with the 95% of samples taken between May and August.

Results

Distribution and Abundance

We examined records of 802 samples distributed throughout the coastal waters of British Columbia, of which 224 contained *N. pungens* (Fig. 1). In the few instances where we found *N. pungens* seaward of the shelf break in depths greater than 200 m, it was usually associated with filaments of shelf water that regularly develop near the coast of Vancouver Island during summer and transport material offshore (Denman et al. 1989). In 1984, we found traces at two stations in the vicinity of Ocean Station P (50°N, 145°W) in the subarctic Pacific (Forbes et al. 1988). *Nitzschia pungens* occurs most frequently off southwest Vancouver Island. It is relatively scarce in the Strait of Georgia and north of Vancouver Island. The maximum concentrations we recorded were 1.06×10^6 cells·L^{-1} in Hecate Strait in early July 1983 and 5.34×10^5 cells·L^{-1} off southwest Vancouver Island in late August 1986.

Nitzschia pungens appears most frequently as a minor component of the large diatom populations that typically develop off the southwest coast of Vancouver Island during late July and August (Mackas et al. 1980; Denman et al. 1981; Mackas and Sefton 1982). While we found it in nearly 60% of all our samples taken in August (Table 1), it occurred in only 29% of samples taken in July, and generally in less than 20% of samples collected in other months. It occasionally becomes the dominant species in the summer blooms. However, in the 20 samples with the highest abundances (Table 2), it ranked first among all species in cell concentration in only four samples. Only 10 samples had concentrations greater than 10^5 cells·L^{-1} and, in the sample ranked twentieth, it comprised fewer than 1% of the phytoplankton cells.

The distribution and abundance of this species encountered on two cruises covering most of the coast during midsummer of 1983 and 1985 (Fig. 2A and 2B) reveals substantial interannual variability. In late June and early July 1983,

TABLE 2. Twenty samples containing the greatest abundances of *N. pungens*, with total phytoplankton cells (cells·L^{-1}), the ratio of the species to the total (by cell number), and its rank in abundance with respect to all other phytoplankton species.

	Latitude (N)	Longitude (W)	Region	*N. pungens*	Total cells	Ratio	Rank
1	53°13.2′	131°02.8′	Hecate St.	1.06×10^6	3.69×10^6	0.29	1
2	53°13.2′	131°02.8′	Hecate St.	6.20×10^5	1.45×10^6	0.43	1
3	48°58.4′	125°47.1′	SW Vancouver Is.	5.34×10^5	6.32×10^5	0.84	1
4	53°13.2′	131°02.8′	Hecate St.	4.34×10^5	1.75×10^6	0.25	1
5	53°11.5′	131°13.6′	Hecate St.	3.36×10^5	1.30×10^6	0.26	2
6	48°39.9′	125°47.4′	SW Vancouver Is.	3.03×10^5	3.40×10^6	0.09	3
7	48°46.2′	126°10.2′	SW Vancouver Is.	1.98×10^5	1.64×10^6	0.12	3
8	53°12.0′	131°02.7′	Hecate St.	1.66×10^5	1.25×10^6	0.13	2
9	53°11.5′	131°13.6′	Hecate St.	1.66×10^5	7.50×10^5	0.22	2
10	48°57.4′	126°25.0′	SW Vancouver Is.	1.12×10^5	1.12×10^7	0.01	17
11	53°11.6′	131°18.6′	Hecate St.	9.48×10^4	5.83×10^5	0.16	2
12	53°11.6′	131°18.6′	Hecate St.	7.68×10^4	6.90×10^5	0.11	3
13	53°11.6′	131°18.6′	Hecate St.	7.15×10^4	5.47×10^5	0.13	2
14	49°18.5′	126°28.7′	W Vancouver Is.	6.90×10^4	4.37×10^6	0.02	9
15	48°24.6′	126°15.8′	SW Vancouver Is.	6.81×10^4	5.45×10^6	0.01	10
16	49°29.5′	126°48.5′	W Vancouver Is.	6.70×10^4	2.30×10^6	0.03	9
17	48°45.3′	125°42.1′	SW Vancouver Is.	4.90×10^4	3.53×10^6	0.01	11
18	53°11.6′	131°00.3′	Hecate St.	4.26×10^4	5.36×10^5	0.08	5
19	48°57.4′	126°25.0′	SW Vancouver Is.	3.95×10^4	3.58×10^6	0.01	25
20	48°49.2′	125°42.0′	SW Vancouver Is.	3.72×10^4	1.26×10^7	0.00	27

virtually no occurrences were observed off the west coast of Vancouver Island, while Hecate Strait contained the highest numbers we have recorded. At the same time of year in 1985, moderate concentrations ($10^3 - 10^5$ cells·L^{-1}) were found at a number of stations along the outer coast of Vancouver Island, but the distribution was relatively sparse and abundances low ($<10^3$ cells·L^{-1}) farther north. Sampling strategy may explain the difference in part. Sampling stations in 1985 were widely separated. In contrast, the 1983 sampling focused in the frontal zone separating shallow, well-mixed water in western Hecate Strait from less-mixed water in the central portion of the strait. This frontal zone characteristically maintains relatively high phytoplankton biomass (Perry et al. 1983) and productivity (Forbes et al. 1986), while biomass is generally more moderate to the east and low to the west. In addition, 1983 was the year with the highest recorded positive temperature anomaly off the west coast of Vancouver Island (Denman et al. 1989), and among the highest temperature anomalies along the coast as a whole (Freeland 1990), associated with the 1982–83 El Niño.

Nitzschia seriata, a species closely related to *N. pungens*, was distinguished on the basis of cell shape and dimensions (Hasle 1965). It was more restricted to the west coast of Vancouver Island and occurred more frequently later in the year than *N. pungens*. The diatom genus *Amphora*, generally benthic (Brunel 1970), was very rare in our samples. If *Amphora coffaeiformis*, which can produce domoic acid (Maranda et al. 1990), was present, concentrations were negligible.

Forms of *N. pungens*

Differentiation of the two forms of *N. pungens*, f. *multiseries* and the nominate form f. *pungens*, is important, as research to date indicates that production of domoic acid in this species is restricted to f. *multiseries* (S. S. Bates, Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6, pers. comm.). Identification and enumeration of all taxa in our samples has been by light microscopy of uncleaned, settled specimens over an inverted microscope. This technique precludes separation of the two forms of the *N. pungens*. The primary difference between the two forms lies in the structure of the intercostal membrane. In *N. pungens* f. *multiseries*, this is perforated by three or four, seldom two, rows of closely spaced poroids 4 – 6 in each 1 μm. In *N. pungens* f. *pungens* there are consistently only two rows of larger poroids, numbering 3–4 in each 1 μm (Hasle 1965). The width in valve view of *N. pungens* f. *multiseries* is also slightly greater and more consistent than in f. *pungens* (4–5 versus 3–4.5 μm). Scanning electron microscopy of a nearshore sample collected at 50°11.2′N, 127°54.5′W, just north of Brooks Peninsula, in August 1989 revealed the presence of both *N. pungens* f. *pungens* and f. *multiseries* (Fig. 3). The identities were confirmed by C. J. Bird (Institute for Marine Biosciences, NRC, Halifax, N.S.) and G. A. Fryxell (Texas A&M University, College Station, TX). Although this preparation was nonquantitative, the nominate form was present in approximately 25 times the concentration of f. *multiseries*.

Associated Water Properties

Frequency distributions normalized to modal frequencies of samples with and without *N. pungens* (Fig. 4) reveal that this species is usually restricted to water temperatures between 8 and 14°C and salinity between 30.0 and 32.5 and to water with relatively high concentrations of chlorophyll and reduced concentrations of dissolved inorganic nutrients, silicate in particular.

We used discriminant analysis (Green and Vascotto 1978) on the log-normalized data to examine whether there are distinctive water properties associated with *N. pungens*. Overall, samples with and without the organism can be discriminated (Wilk's $\Lambda = 0.686$, Rao's $F = 34.00$ with df = 7, 520: $p < 0.01$) (Takeuchi et al. 1982). Univariate F tests for the dependent variables indicate that salinity, temperature, chlorophyll *a*, nitrate plus nitrite, and reactive silicate (all with $p < 0.01$) are the principal contributors to this separation, while depth and phosphate concentration offer little ($p > 0.2$ and 0.5, respec-

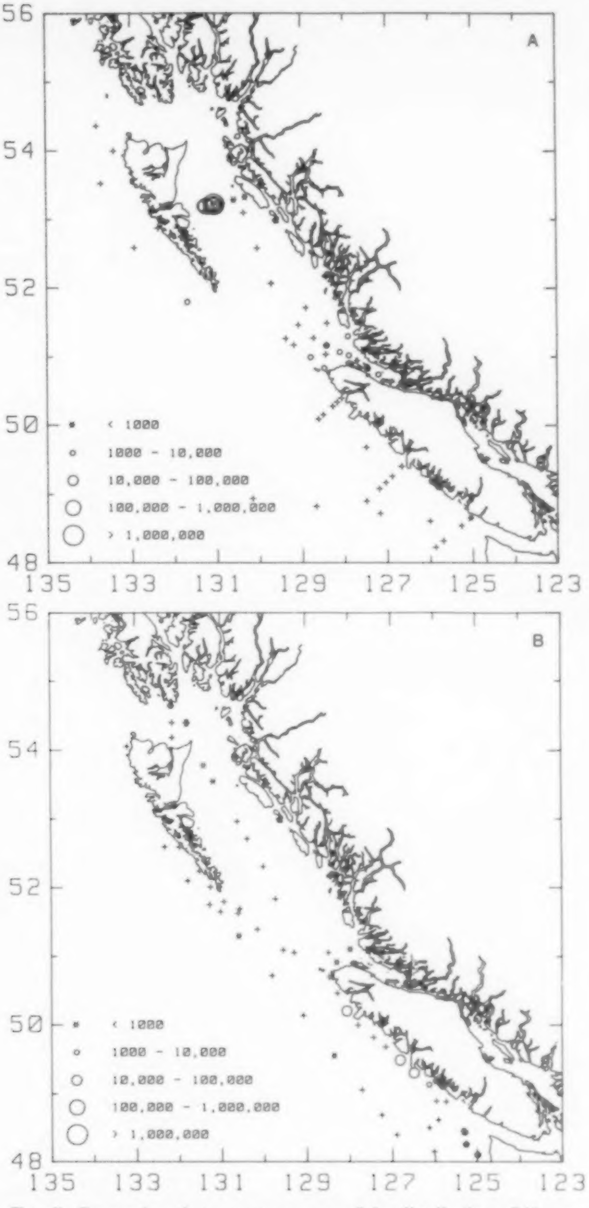

FIG. 2. Comparison between two years of the distribution of *N. pungens* along the British Columbia coast in early summer. Samples containing *N. pungens* are indicated by circles representing abundances (cells·L^{-1}) or an asterisk where there were fewer than 1000 cells·L^{-1}. A plus sign indicates samples that did not contain this species. (A) Samples collected 28 June – 10 July 1983; (B) samples collected 24 June – 11 July 1985.

tively). The frequency distribution of discriminant scores (Fig. 5) shows that there is substantial overlap, however. This suggests that the utility of the discriminant function for predicting presence or absence of *N. pungens* is restricted. When actual group membership is tabulated against predicted membership, based on discriminant scores (Table 3), it can be seen that the discriminant function performs well in predicting correctly that the species is absent (297 out of 342: 87%). However, there is a strong possibility of concluding that it is not present (78 out of 185: 42%) when in fact it is.

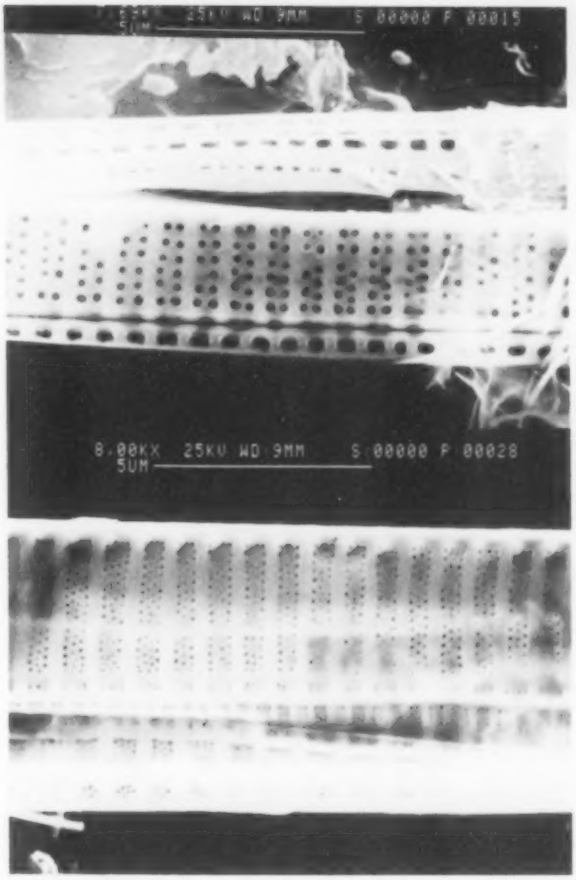
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FIG. 3. Scanning electron micrographs of specimens of *N. pungens* collected 13 August 1989 at 50°11.2′N, 127°54.8′W. Top: *N. pungens* f. *pungens*; bottom: *N. pungens* f. *multiseries*.

Discussion

Reviewing records of *N. pungens*, Hasle (1972) found it to be common between 40°N and 40°S in the Atlantic and Pacific oceans, occasionally farther north in the Atlantic, and almost exclusively in continental shelf waters. A temperature range from 2 to 28°C and salinity range from 30 to 36, 24 in one instance, were indicated. Among more recent records, Marshall and Cohn (1983, 1987) found this species in all seasons along the Atlantic coast of the United States. There it forms an important constituent of the phytoplankton community during the spring bloom, which may occur as early as December, and during the summer. At other times of the year, its abundance is limited. *Nitzschia pungens* occurs fairly regularly in inshore waters of the Bay of Fundy from the end of June to early October (Wildish et al. 1988). In the Pacific, it is found in Korean coastal waters (Yoo and Lee 1982) and in upwelling areas around the Izu island chain, southeast of Japan (Furuya et al. 1986), as well as the areas known earlier off southern California, Baja California, and in the Gulf of California (Cupp 1943). It has also been recorded in the Mediterranean (Cahet et al.

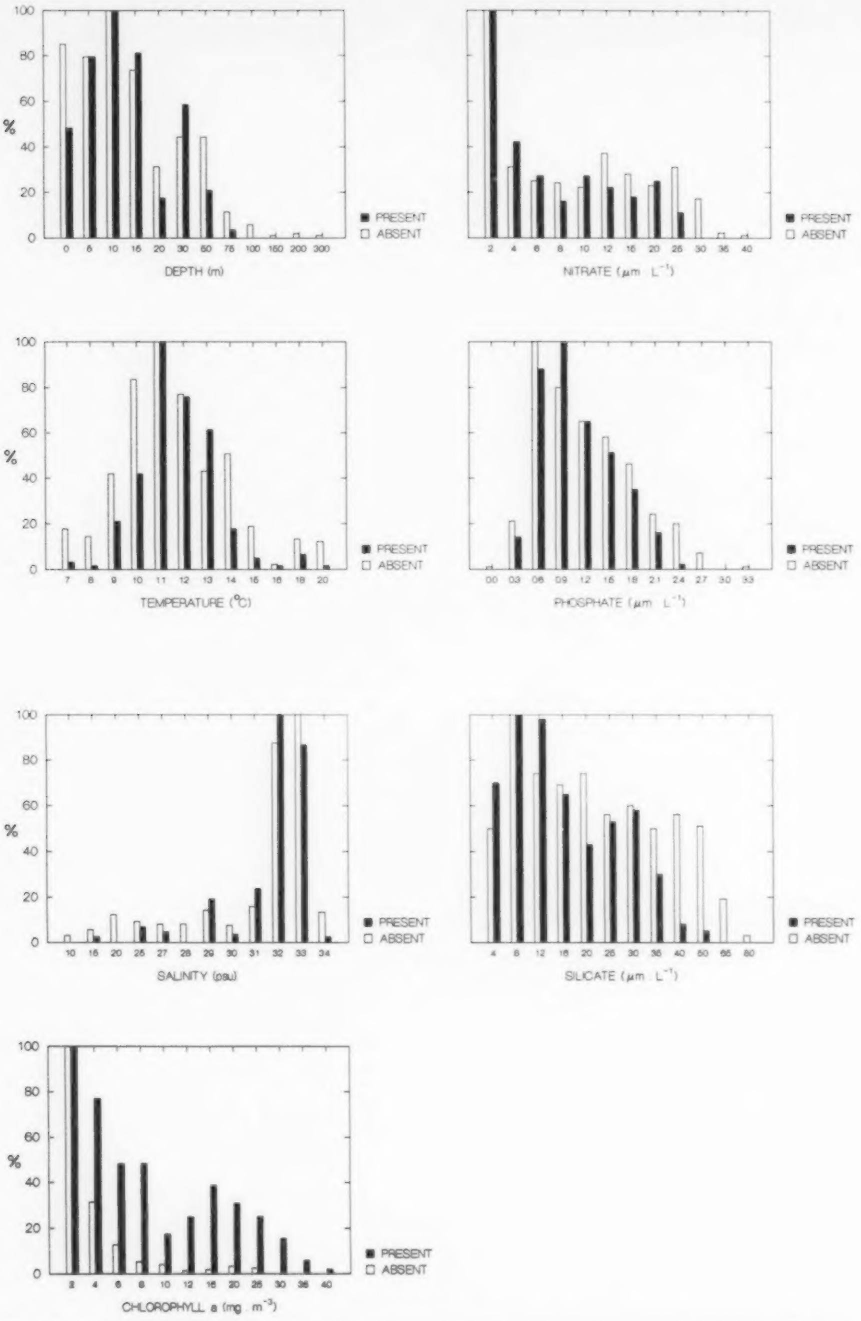

FIG. 4. Frequency distributions of samples where *N. pungens* was present or absent, normalized to modal frequencies, for depth, temperature, salinity, chlorophyll *a*, reactive nitrate plus nitrite, phosphate, and silicate.

1972; Neveux et al. 1975; Fiala et al. 1976) and Caribbean (Navarro 1983) seas, in the South Atlantic Ocean along the coast of Brazil (Gonzalez-Rodriguez et al. 1985), and in the Indian sector of the Southern Ocean as far south as 65°S (Kawamura and Ichikawa 1984).

Few studies provide details of the identity and distribution of marine phytoplankton of British Columbia, and most of these have refrained from specifying members of the *Pseudonitzschia* group. Shim (1977) followed one annual cycle of species composition and abundance at five stations in the Strait of Georgia and Juan de Fuca Strait. He found *N. pungens* throughout the year, with a maximum abundance in July. In sediment trap

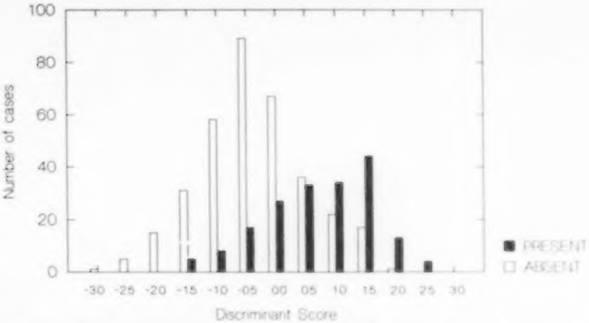

FIG. 5. Frequency distribution of the discriminant scores of samples where *N. pungens* was present or absent.

TABLE 3. Table of actual (rows) by predicted (columns) frequencies determined by discriminant scores: the analysis correctly predicts 297 samples with *N. pungens* absent, when there are actually 342 with it absent, and incorrectly predicts absence in 78 of the 185 samples that actually do contain it.

	Predicted		
	Absent	Present	Total
Actual			
Absent	297	45	342
Present	78	107	185
Total	375	152	527

collections from Saanich Inlet on southeastern Vancouver Island from November 1984 to November 1985, Sancetta and Calvert (1988) found it in all months except March and April. It was always rare (2–5% of diatom frustules) or very rare (<2%). Over a 3-yr period, a more general pattern appeared, with *N. pungens* a characteristic minor component of the summer (July–August) period, both in Saanich Inlet and in Jervis Inlet, on the south coast of the mainland (Sancetta 1989). Also in Saanich Inlet, this species developed as one of the dominant diatoms, under some conditions, in enclosed water column experiments initiated in late August (Eppley et al. 1978). There have been no detailed taxonomic studies of phytoplankton off the west coast of Vancouver Island or in northern British Columbian coastal waters, although Taylor and Waters (1982) have provided data from the subarctic Pacific west of the continental shelf. They did not record *N. pungens*. Although two samples collected in 1984 from Ocean Station P, in the subarctic gyre of the eastern Pacific Ocean, contained traces of *N. pungens* (Forbes et al. 1988), it was not found at all in 1987 in samples from the area of Station P and further north, in the vicinity of 52°N, 143°W (J. R. Forbes, unpubl. data).

As few studies have distinguished f. *multiseries* from the nominate form of the species, it is difficult to get a sense of the relative distribution of each. The original description of f. *multiseries* (Hasle 1965) was based on samples from Drøbak in Oslofjord, Norway, the Atlantic (Chesapeake Bay) and Pacific (Oregon) coasts of the United States, Atlantida, Uruguay, and Quequen, Argentina. Apart from those associated with the domoic acid investigation in eastern Canada (Subba Rao et al. 1988; Bates et al. 1989; Bates and Worms 1989), the only other records of f. *multiseries* are from the Gulf of Mexico (Hasle 1972; Fryxell et al. 1990), Korea (Fryxell et al. 1990), and Japan (Takano and Kuroki 1977). Hasle (1965) commented that f. *multiseries* appeared seasonally in Oslofjord during autumn and early winter (September–February) in water temperatures from 2 to 14°C and, on one occasion, −1.4°C. This is consistent with records in the coastal embayments of northern and eastern Prince Edward Island, where blooms of f. *pungens* have occurred during the early part of August, but f. *multiseries* has not appeared until late August. The proportion of f. *multiseries* then generally increased through the fall (Smith et al. 1990), with f. *multiseries* persisting in substantial concentrations as late as mid-January and with traces found throughout the winter, under ice cover (Smith et al. 1989). The overall distributional data for the two forms suggests that f. *multiseries* may be more characteristic of colder temperatures than the nominate form. However, it is not excluded even from tropical waters, as the samples from the Gulf of Mexico illustrate (Hasle 1972; Fryxell et al. 1990). Along the British Columbia coast, the only information apart from this study is the electron micrograph of a sample (undated) from the Strait of Georgia by Shim (1976), which shows the nominate form. While f. *multiseries* appears in the summer here, we postulate that samples during cooler seasons and from mainland inlets with glacial inflow, such as Alice Arm, are more likely to contain a higher proportion than we observed in our summer sample.

If the abundance of *N. pungens* in our records is examined with respect to some of the environmental variables, several pertinent details emerge. With one exception, all samples with cell concentrations greater than 2×10^5 cells·L^{-1} occurred at a depth greater than 20 m, although the modal depth for species presence was 10 m (Fig. 4). This suggests that the development of high cell concentrations may be inhibited by elevated irradiance, or that these high concentrations appear in relatively senescent populations aggregating by sinking near the base of the upper mixed layer. While Furuya et al. (1986) found that it was numerically the dominant species in regional upwelling of the Kuroshio current around the Izu islands, over a broader area of the western North Pacific, Furuya and Marumo (1983) found it regularly in the subsurface (approximately 50 m) chlorophyll maximum.

None of our samples with high cell concentrations were fully depleted in nitrogen or silicate sources. Carbon uptake measurements at the station with the highest cell density showed substantial photosynthetic capacity (P_m^B = 7.3 mg C·(mg Chl *a*)$^{-1}$·h^{-1}) and uptake under light-limited condition (α = 0.061 mg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEin·m^{-2}·s^{-1})$^{-1}$), indicating a strong potential for additional growth. The range of P_m^B in Hecate Strait, during the cruise in which this sample was collected, was 3.5–17.5 mg C·(mg Chl *a*)$^{-1}$·h^{-1}; the range of α was 0.028–0.140 mg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEin·m^{-2}·s^{-1})$^{-1}$ (Forbes et al. 1986). The maximum was the second highest value we have recorded along the British Columbia coast. More typical values for P_m^B range from 0.5 to < 10 mg C·(mg Chl *a*)$^{-1}$·h^{-1} and for α from 0.01 to 0.05 mg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEin·m^{-2}·s^{-1})$^{-1}$ (Forbes et al. 1983, 1987). The nitrate plus nitrite concentration at the station with maximum abundance was 4.2 μmol·L^{-1}. As a crude estimate, using 70 pg C·cell^{-1} (Bates et al. 1989) and a typical C:N ratio of 6.5 (Valiela 1984), this should permit at least a fivefold increase in cell density to approximately 5×10^6 cells·L^{-1}. If the cells were producing domoic acid, advection of the bloom to shore would have had the potential to cause unacceptable contamination of shellfish. Hanic and Cormier (1989) estimated that a concentration of 2×10^5 to 4×10^5 cells·L^{-1} over a period of 3–4 wk is sufficient to produce 20 $\mu g \cdot g^{-1}$ domoic acid in edible tissue of mussels, the current regulatory limit in Canada.

Many cells in the dense samples collected in August 1986 off the southwest coast of Vancouver Island were in poor condition, and there were many empty (uncounted) frustules, indicating that the population was in a senescent phase. High levels of available nitrate (17.6 $\mu mol \cdot L^{-1}$) remained, however. If the population were f. *multiseries*, the conditions that preliminary studies (de Freitas et al. 1989) indicate necessary for significant domoic acid production would be met.

We have found *N. pungens* to be widely distributed and show considerable interannual variability in distribution and abundance. Prediction of presence from discriminant analysis of existing environmental variables is questionable, but has potential. Future assessment of the risk of domoic acid contamination in British Columbia waters should establish whether or not *N. pungens* produces domoic acid in this region and determine the distribution of both the nominate form and f. *multiseries*. Also to be addressed are whether high abundances over the shelf are reflected in or followed by high abundances near shore and whether prediction based on environmental variables can be made more powerful.

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References

Bates, S. S., C. J. Bird, A. S. W. de Freitas, R. Foxall, M. Gilgan, L. A. Hanic, G. R. Johnson, A. W. McCulloch, P. Odense, R. Pocklington, M. A. Quilliam, P. G. Sim, J. C. Smith, D. V. Subba Rao, E. C. D. Todd, J. A. Walter, and J. L. C. Wright. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish. Aquat. Sci. 46: 1203–1215.

Bates, S. S., and J. Worms [ed.] 1989. Proceedings of the first Canadian workshop on harmful marine algae. Gulf Fisheries Centre, Moncton, N.B., September 27–28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1712: 58 p.

Brunel, J. 1970. Le phytoplancton de la baie des Chaleurs. 2^e éd. Les Presses de l'Université de Montréal, Montréal (Québec). 365 p.

Cahet, G., M. Fiala, G. Jacques, and M. Panouse. 1972. Production primaire au niveau de la thermocline en zone néritique de Méditerranée nord-occidentale. Mar. Biol. 14: 32–40.

Cupp, E. E. 1943. Marine planktonic diatoms of the west coast of North America. Bull. Scripps Inst. Oceanogr. 5: 1–238.

Debonnel, G., L. Beauchesne, and C. de Montigny. 1989. Domoic acid, the alleged "mussel toxin," might produce its neurotoxic effect through kainate receptor activation: an electrophysiological study in the dorsal hippocampus. Can. J. Physiol. Pharmacol. 67: 29–33.

de Freitas, A., S. S. Bates, R. Pocklington, and J. L. C. Wright. 1989. Domoic acid: some biological and ecological considerations on the production of this neurotoxic secondary metabolite by the diatom *Nitzschia pungens* (abstract), p. 12. *In* S. S. Bates and J. Worms [ed.] Proceedings of the first Canadian workshop on harmful marine algae. Gulf Fisheries Centre, Moncton, N.B., September 27–28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1712: 58 p.

Denman, K., R. Forbes, D. Mackas, S. Hill, and H. Sefton. 1985. Ocean ecology data report: British Columbia coastal waters, 29 June–10 July 1983. Can. Data Rep. Hydrogr. Ocean Sci. 36: 77 p.

Denman, K. L., H. J. Freeland, and D. L. Mackas. 1989. Comparison of time scales for biomass transfer up the marine food web and coastal transport processes, p. 255–264. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Publ. Fish. Aquat. Sci. 108.

Denman, K. L., D. L. Mackas, H. J. Freeland, M. J. Austin, and S. H. Hill. 1981. Persistent upwelling and mesoscale zones of high productivity off the west coast of Vancouver Island, Canada, p. 514–521. *In* F. A. Richards [ed.] Coastal upwelling. American Geophysical Union, Washington, DC.

Department of Fisheries and Oceans (Canada). 1988. British Columbia commercial catch statistics 1988.

Eppley, R. W., P. Koeller, and G. T. Wallace Jr. 1978. Stirring influences the phytoplankton species composition within enclosed columns of coastal sea water. J. Exp. Mar. Biol. Ecol. 32: 219–239.

Fiala, M., G. Cahet, G. Jacques, J. Neveux, and M. Panouse. 1976. Fertilisation de communautés phytoplanctoniques. 1. Cas d'un milieu oligotrophe: Méditerranée nord-occidentale. J. Exp. Mar. Biol. Ecol. 24: 151–163.

Forbes, J. R., S. L. Buckingham, and A. T. Earmme. 1987. Phytoplankton productivity experiments in British Columbia coastal waters, 1986. Can. Data Rep. Hydrogr. Ocean Sci. 56: 169 p.

Forbes, J. R., K. L. Denman, and D. L. Mackas. 1986. Determinations of photosynthetic capacity in coastal marine phytoplankton: effects of assay irradiance and variability of photosynthetic parameters. Mar. Ecol. Prog. Ser. 32: 181–191.

Forbes, J. R., K. L. Denman, D. L. Mackas, and R. M. Brown. 1988. Ocean ecology data report: subarctic Pacific Ocean, May 1984 (Project SUPER). Can. Data Rep. Hydrogr. Ocean Sci. 64: 147 p.

Forbes, J. R., H. A. Sefton, and K. de Macedo. 1983. Phytoplankton productivity experiments in British Columbia coastal waters, 1982 and 1983. Can. Data Rep. Hydrogr. Ocean Sci. 14: 193 p.

Freeland, H. J. 1990. Sea surface temperatures along the coast of British Columbia: regional evidence for a warming trend. Can. J. Fish. Aquat. Sci. 47: 346–350.

Fryxell, G. A., M. E. Reap, and D. L. Valencic. 1990. *N. pungens* Grun. f. *multiseries* Hasle: observations of a known neurotoxic diatom. Nova Hedwigia Beih. 100: 171–188.

Furuya, K., and R. Marumo. 1983. The structure of the phytoplankton community in the subsurface chlorophyll maximum in the western North Pacific Ocean. J. Plankton Res. 5: 393–406.

Furuya, K., M. Takahashi, and T. Nemoto. 1986. Summer phytoplankton community structure and growth in a regional upwelling area off Hachijo Island, Japan. J. Exp. Mar. Biol. Ecol. 96: 43–55.

Gonzalez-Rodriguez, É., S. Y. Maestrini, J. L. Valentin, and D. Rivera-Tenebaum. 1985. Variation de la composition spécifique du phytoplancton de Cabo Frio cultivé en présence d'enrichissements différentiels. Oceanol. Acta 8: 441–452.

Green, R. H., and G. L. Vascotto. 1978. A method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. Water Res. 12: 583–590.

Hanic, L. A., and R. Cormier. 1989. *Nitzschia pungens* and mussel-domoic acid concentrations in eastern Prince Edward Island, November 1988 – February 1989 (abstract), p. 26. *In* S. S. Bates and J. Worms [ed.] Proceedings of the first Canadian workshop on harmful marine algae. Gulf Fisheries Centre, Moncton, N.B., September 27–28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1712: 58 p.

Hasle, G. R. 1965. *Nitzschia* and *Fragilariopsis* species studied in the light and electron microscopes. II. The group *Pseudonitzschia*. Skr. Utgitt Nor. Vidensk-Akad. Oslo I. Mat.-Naturvidensk. Kl. NY Ser. No. 18: 1–29.

1972. The distribution of *Nitzschia seriata* CLEVE and allied species. Nova Hedwigia Beih. 39: 171–190.

1978. Settling. The inverted microscope method, p. 88–96. *In* A. Sournia [ed.] Phytoplankton manual. UNESCO, Paris.

House of Commons (Canada). 1988. Aquaculture in Canada. Report of the Standing Committee on Fisheries and Oceans. 129 p.

Kawamura, A., and T. Ichikawa. 1984. Distribution of diatoms in a small area in the Indian sector of the Antarctic. Mem. Nat. Inst. Polar Res. (Tokyo), Spec. Issue No. 32: 25–37.

Mackas, D. L., G. C. Louttit, and M. J. Austin. 1980. Spatial distribution of zooplankton and phytoplankton in British Columbia coastal waters. Can. J. Fish. Aquat. Sci. 37: 1476–1487.

Mackas, D. L., and H. A. Sefton. 1982. Plankton species assemblages off southwestern Vancouver Island: geographic pattern and temporal variability. J. Mar. Res. 40: 1173–1200.

Maranda, L., R. Wang, K. Masuda, and Y. Shimizu. 1990. Investigation of the source of domoic acid in mussels, p. 300–304. *In* E Graneli, B. Sundström, L. Edler, and D. M. Anderson [ed.] Toxic marine phytoplankton. Elsevier, New York, NY.

Marshall, H. G., and M. S. Cohn. 1983. Distribution and composition of phytoplankton in northeastern coastal waters of the United States. Estuarine Coastal Shelf Sci. 17: 119–131.

1987. Phytoplankton distribution along the eastern coast of the USA. Part VI. Shelf waters between Cape Henry and Cape May. J. Plankton Res. 9: 139–149.

Navarro, J. N. 1983. A survey of the marine diatoms of Puerto Rico. 7. Suborder Raphidaceae: Families Auriculaceae, Epithemiaceae, Nitzschiaceae and Surirellaceae. Bot. Mar. 26: 393–408.

Neveux, J., M. Fiala, G. Jacques, and M. Panouse. 1975. Phytoplancton et matériel particulaire à Banyuls-sur-Mer (Golfe du Lion), 1973. Vie Milieu (Ser. B Oceanogr.) 25: 85–98.

Perl, T. M., L. Bedard, T. Kosatsky, J. C. Hockin, E. Todd, and R. S.. Remis. 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. N. Engl. J. Med. 322: 1775–1780.

Perry, R. I., B. R. Dilke, and T. R. Parsons. 1983. Tidal mixing and summer plankton distributions in Hecate Strait, British Columbia. Can. J. Fish. Aquat. Sci. 40: 871–887.

Sancetta, C. 1989. Spatial and temporal trends of diatom flux in British Columbia fjords. J. Plankton Res. 11: 503–520.

Sancetta, C., and S. E. Calvert. 1988. The annual cycle of sedimentation in Saanich Inlet, British Columbia: implications for the interpretation of diatom fossil assemblages. Deep-Sea Res. 35: 71–90.

Shim, J. H. 1976. Distribution and taxonomy of planktonic diatoms in the Strait of Georgia, British Columbia. Ph.D. thesis, University of British Columbia, Vancouver, B.C. 272 p.

1977. A taxonomic study of marine plankton diatoms of Vancouver Island coastal waters. Proc. Coll. Nat. Sci. SNU 2(2): 79–184.

Smith, J. C., R. Angus, K. Pauley, P. Cormier, S. Bates, L. Hanic, J. Worms, and T. Sephton. 1989. Toxic blooms of the domoic acid-containing diatom *Nitzschia pungens* in eastern Prince Edward Island in the fall of 1988 and late summer of 1989 (abstract), p. 11. *In* S. S. Bates and J. Worms [ed.] Proceedings of the first Canadian workshop on harmful marine algae. Gulf Fisheries Centre, Moncton, N. B., September 27–28, 1989. Can Tech. Rep. Fish. Aquat. Sci. 1712: 58 p.

Smith, J. C., P. Odense, R. Angus, S. Bates, C. Bird, P. Cormier, A. de Freitas, C. Leger, D. O'Neil, and K. Pauley. 1990. Domoic acid levels in field and laboratory populations of *Nitzschia pungens* forma *multiseries* and *Nitzschia pungens* forma *pungens*. Bull. Aquacult. Assoc. Can. 90(4): 27–31.

Subba Rao, D. V., M. A. Quilliam, and R. Pocklington. 1988. Domoic acid — a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. Can. J. Fish. Aquat. Sci. 45: 2076–2079.

Takano, H., and K. Kuroki. 1977. Some diatoms in the section *Pseudonitzschia* in the coastal waters of Japan. Bull. Tokai Reg. Fish. Res. Lab. 91: 41–51.

Takeuchi, K., H. Yanai, and B. N. Mukherjee. 1982. The foundations of multivariate analysis. Wiley Eastern Ltd., New Delhi. 458 p.

Taylor, F. J. R., and R. E. Waters. 1982. Spring phytoplankton in the subarctic North Pacific Ocean. Mar. Biol. 67: 323–335.

Throndsen, J. 1978. Preservation and storage, p. 69–74. *In* A. Sournia [ed.] Phytoplankton manual. UNESCO, Paris.

Valiela, I. 1984. Marine ecological processes. Springer-Verlag, New York, NY. 546 p.

Wildish, D. J., J. L. Martin, A. J. Wilson, and A. M. DeCoste. 1988. Environmental monitoring of the Bay of Fundy salmonid mariculture industry during 1986 and 1987. Can. Tech. Rep. Fish. Aquat. Sci. 1648: 44 p.

Yoo, K. I., and J. H. Lee. 1982. Studies on the planktonic diatoms in the vicinity of Kori nuclear power plant. Bull. Korea Ocean Res. Dev. Inst. 4: 53–62.

Measurement of Scope for Change in Ascendency for Short-Term Assessment of Community Stress

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Scope for growth, or the resource balance of an organism or population, has been used as a comparative descriptor for the short-term assessment of stress effects. This concept may be extended to all levels of biological organization, and in particular to the assessment of early responses of communities to anthropogenic stress (or subsidy). The size (throughput) and development (organization) of a biological system can be described by the state function ascendency. We define the scope for change in ascendency of a biological system as the difference between the ascendency of inputs and the ascendency of outputs. This balance increases when a resource subsidy is used by the system to build size and organization, and decreases under stress. Changes in a system's resource balance and distribution in terms of flows help trace effects at one level to effects at the other. Computer simulations of an epilimnion community indicated that scope for change in ascendency gives a sensitive and fast response to perturbations. This index may provide a means for short-term assessment of anthropogenic stress, or management options.

L'évolution à court terme du bilan énergétique (« scope for growth ») d'un organisme ou d'une population a été utilisée pour évaluer les effets dus au stress. Ce concept peut être étendu à tous les niveaux d'organisation biologique, et en particulier à l'estimation de la réponse initiale d'une communauté à un stress ou à un subside anthropogènes. Dimension (débit) et développement (organisation) d'un système biologique peuvent être décrits par une fonction d'état (« ascendency »). Nous définissons le bilan énergétique d'un système biologique (« scope for change in ascendency ») par la différence entre dimension et développement des flux entrants et sortants. Cet indice augmente lorsqu'un subside de ressources est utilisé par le système pour accroître sa dimension et son organisation, et diminue sous l'effet d'un stress. Les changements dans le bilan et la distribution énergétiques des flux des ressources permettent de retracer les effets d'un niveau d'organisation à un autre. D'après des simulations sur ordinateur d'une communauté épilimnétique, l'indice de bilan énergétique paraît sensible aux perturbations. Cet indice permettrait d'évaluer à court terme les effets dus aux stress anthropogènes, ou d'évaluer les options en matière de gestion de l'environnement.

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The ultimate aim of environmental management is to perform at the community and ecosystem level. However, there is a relative lack of understanding of stress effects on communities as compared with populations, organisms, or cells. Reciprocally, knowledge of effects at these finer levels of resolution is difficult to integrate into an understanding of the behavior of a community under stress: as often stated in systems ecology, the whole behaves as more than the sum of its parts. Thus, there is a need for management approaches for communities or ecosystems, but presently there is a broad gap between theory and practice. Nonetheless, macroscopic concepts can promote a better understanding of perturbations of ecosystems and suggest management schemes, and help evaluate them (Ulanowicz and Platt 1985; Emery and Mattson 1986). Here we propose a concept to help guide environmental management at all levels of biological organization, and we emphasize its use for communities. Specifically, we define a descriptor of "stress" in the biotic component of the ecosystem, the community, that should be sensitive to stress or subsidy in the short term and might be used to predict long-term changes caused by stress or subsidy. This concept derives from the use of scope for growth as an indicator of stress on organisms, on one hand, and from network analysis of ecosystems, on the other. Although it may be applied to any management scheme, we shall refer to the ecotoxicology of freshwater communities to illustrate its principle.

Scope for growth (SfG), or the energy incorporated into somatic growth and gamete production (Warren and Davis 1967), reflects an organism's energy balance (Winberg 1960). SfG increases when there is more energy available for growth, and decreases when the organism is under stress and is using its reserves for maintenance metabolism. A reduced SfG is a generalized, early response to environmental stresses, and will have detrimental effects (Bayne et al. 1978; Gilfillan 1980). Thus, this index provides an estimate not only of physiological condition, but also of the ecological consequences of stress. Calow (1989) used this approach with populations, by measuring a population's SfG from the energy acquired and the respiratory and excretory losses. He showed that a response to stress may occur through changes in SfG and in population size. Moreover, SfG can be correlated quantitatively with changes in the subprocesses of intake, assimilation, respiration, growth, or reproduction. The use of SfG allows for comparisons between populations, between species, or between toxicants or their concentrations. SfG may be a good descriptor of population status in terms of "health" or "stress," with predictive value as to the magnitude of response.

This approach may be extended to all levels of biological organization. From subcellular structures to ecosystems, biological processes appear as networks of flows of resources and energy through transformers, each network being embedded in the next-level network (Ulanowicz and Kemp 1979; H. T. Odum 1985). Allocation pathways and trade-off options are subjected to selection, and are affected by natural and anthropogenic stresses (Wiens 1984; Calow 1989).

Moreover, we may take into account not only quantitative growth (increase in size), but also qualitative development (increase in organization). These processes are often occurring simultaneously. Hochachka and Somero (1984), for example, spoke of cellular "growth and integration." Indeed, responses to perturbations may result in no apparent change in size, and yet cause significant changes in organization. For instance, addition of cadmium to a lake did not affect total community biomass or production, but it did affect species composition (Schindler 1987). We shall therefore refer to "growth and development" of a cell, a tissue, an organ, an organism, a population, or a community. The size and organization of any system can be characterized by a single state variable, ascendency (Ulanowicz 1986), which tends to increase through growth and development. Ascendency has the units of total system throughput of a resource in the system, and information content, and serves as a measure of the system's degree of self-organization relative to its trophic capacity and limitation. Ascendency has the form

$$A = \sum_{j=0}^{n} \sum_{i=1}^{n+2} T_{ji} \, log \frac{T_{ji}T}{T_{j\cdot}T_{\cdot i}}$$

where T is the system's throughput of a resource, T_{ji} are resource flows from donor compartments j to receiver compartments i in the network, $T_{j\cdot}$ are total compartment outputs and $T_{\cdot i}$ total compartment inputs. This description refers to an open network with n internal compartments, where compartment 0 is the source of all exogenous inputs into the system, compartment $n + 1$ is the sink for exports, and compartment $n + 2$ is the sink for dissipative losses (Hirata and Ulanowicz 1984). Its applications include nonsteady-state systems, which makes it useful for understanding system perturbations. The dimension of ascendency is flow·bits, where flows can be energy or matter, e.g. joule·bits per square metre per year or milligrams of nutrient·bits per square metre per year for a community.

Ascendency encompasses the notions of system "size" (number of compartments and throughput) and "development" (information inherent in the network connection pattern). When communities are free from unusual external perturbations, certain developmental trends are observable. Disturbance tends to arrest or reverse such autogenic development and to produce changes in energetics, nutrient cycling, and community structure and function (E. P. Odum 1969, 1985; Margalef 1981), and hence in ascendency (Ulanowicz 1986).

Scope for Change in Ascendency

By anology to SfG, but now with reference to any biological system, from cells to communities, we propose to examine the concept of "scope for change in ascendency" (SfCA) or, synonymously, "scope for growth and development," to estimate "instantaneous" changes in the balance of flows. Such changes may be early responses to disturbance that will translate in the longer term into changes in system ascendency.

We define SfCA as the difference between ascendency of inputs and ascendency of outputs, taking into account all internal, living compartments. These two terms can be derived from the system's ascendency (see Appendix). This formulation of SfCA is preferable to the one that we have proposed before (Genoni and Pahl-Wostl 1990), which only accounted for the magnitude of inputs and outputs, as in SfG. Expressing SfCA as the difference between input and output ascendency implies that the response to a disturbance does not depend only on the magnitude of the change in the resource flow. Rather, it also depends on where in the network these changes are occurring, and on changes in the network topology. Thus:

$$\text{SfCA} = \text{Ascendency of inputs} - \text{Ascendency of outputs}$$
$$= \sum_{j=0}^{n} \sum_{i=1}^{n} \frac{T_{ji}}{2} \log \frac{T_{ji}T}{T_{\cdot i}^{\,2}} - \sum_{j=1}^{n} \sum_{i=1}^{n+2} \frac{T_{ji}}{2} \log \frac{T_{ji}T}{T_{j\cdot}^{\,2}}$$

Using for illustration the average yearly values from the classic example of the Cone Spring ecosystem (Fig. 1), this calculation takes the form

$$\text{Community SfCA} = \left(\frac{11184}{2} \log \frac{11184 \cdot 42445}{11184^2}\right) + \left(\frac{2309}{2} \log \frac{2309 \cdot 42445}{2384^2}\right) + \ldots$$
$$= 2751.7 \text{ kcal}\cdot\text{bits}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$$
$$(= 1151.7 \times 10^4 \text{ J}\cdot\text{bits}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}).$$

This "steady-state" value is positive, even where inputs and outputs are in balance, because communities, like all biological systems, are dissipative structures. The very flow of resources in the system maintains a high state of internal organization and thereby maintains a potential for further change (H. T. Odum 1985). Scope for change in ascendency increases when a resource subsidy is used by the system to build or maintain size and organization, and decreases under stress, when resources are being lost, or, in the words of Margalef (1981), "leaked" by the system. In a natural system, a fluctuation of SfCA may be expected to occur in time. For measuring natural short-term changes in SfCA or changes in SfCA caused by perturbations, a sampling of resource flows at short time intervals would afford the temporal resolution desired.

Stress would first show as a depression of SfCA. Thus, SfCA provides a comparative index for evaluation of effects of toxicants, acid precipitation, eutrophication, fishery exploitation, or introduction of exotic species. Moreover, it allows comparing biological systems in different stages of development, as these may differ in their response to stress (Odum 1981; Rapport et al. 1985). Conversely, a subsidy would first show as an increase in SfCA. Thus, another possible application may be the comparison of the health status of a system before and after application of a management plan. For example, sewage treat-

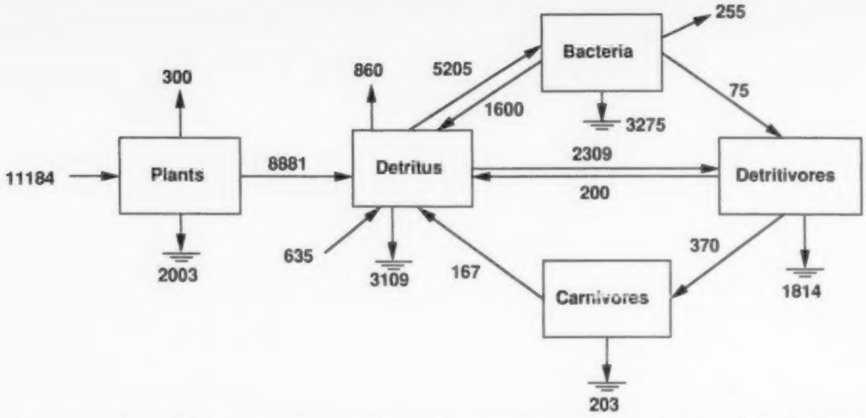

FIG. 1. The Cone Spring ecosystem. Energy flows are expressed in units of kcal·m^{-2}·yr^{-1}. (From R. E. Ulanowicz. 1986. Identifying the structure of cycling in ecosystems. Mathematical biosciences. Vol. 65. Used with permission of Elsevier, Amsterdam, The Netherlands.)

ment may be expected to produce an increase in the SfCA of a lake or stream.

It is possible to keep track of changes occurring at the next lower level of resolution (for a community, its populations), and there are several reasons for doing so. A system's stability and resilience properties emerge largely from the nature and interactions of its parts. Stress or subsidies affect all biological levels, with the effects at "lower" levels causing or interacting with those at "higher" levels (E. P. Odum 1985; Rapport et al. 1985). Thus, stresses that affect populations are likely to affect community SfCA and, eventually, ecosystem ascendency. Ultimately, environmental management is likely to rely on an integration of the understanding of stress effects at the various "hierarchical" levels, and to predict responses at higher levels from responses at lower levels. The SfCA approach allows comparing changes occurring at different hierarchical levels. The whole being more than the sum of its parts, the topology resulting from the interaction of various populations would imply, for example, a community SfCA different from the mere sum of the population SfCA's. The compounded effect, on the community SfCA, of a disturbance on populations would likely be modified by the additional complexity resulting from the interaction between populations (Hirata and Ulanowicz 1985). Measuring SfCA at both levels would tell how much of a change in community SfCA can be explained by changes in populations, and how much by the interactions between populations.

Simulations

To investigate the changes induced in a community by hypothetical toxicants affecting selected population parameters, we performed computer simulations. Kooijman and Metz (1984) used a similar approach for a population model. We adapted a validated simulation model of a temperate mesotrophic lake (Lake Ontario: Scavia 1980) to the MacMETH simulation environment (Wirth et al. 1988) on an Apple MacIntosh II personal computer. The simulation program consisted of a set of differential equations describing population processes and carbon flows between populations. We reduced the complexity of the original model by retaining only the epilimnetic components to the exclusion of mysids. Thus the model included three functional categories of phytoplankton (all diatoms, other small algae, and other large algae, in Scavia's model), three of zooplankton (small cladocerans, large cladocerans, and herbivorous copepods), and that of omnivores. The three categories of phytoplankton differed in optimal light intensity, optimal temperature, and nutrient saturation constants for inorganic phosphorus and for nitrate. The three categories of zooplankton differed in optimal temperature, maximal grazing rate, feeding saturation constant, feeding threshold, grazing preferences, feeding efficiency, and excretion rates. We added a bacterial compartment. In order to simulate perturbations by toxicants, we altered selected parameters in the program's differential equations. Community ascendency and scope for change in ascendency were calculated for short (2-d) time intervals and expressed in mass of carbon per projected surface area of epilimnion, i.e. integrated over the entire epilimnion depth.

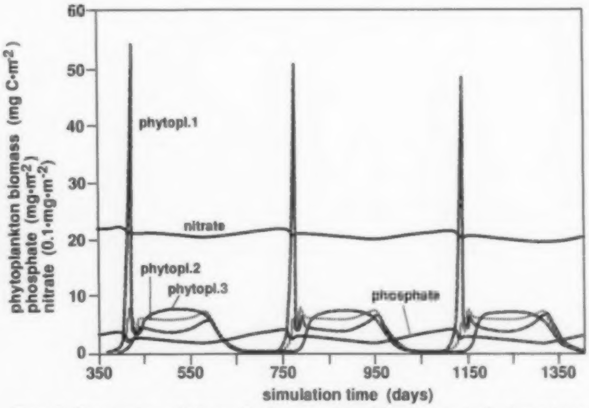

FIG. 2. Dynamics of phytoplankton carbon biomasses and of nutrients (inorganic phosphate and nitrate) over 3 yr in the simulation model. Zooplankton, omnivores, and bacteria are not shown, for clarity.

A standard run showed, from the second simulation year on, the spring and summer algal blooms characteristic of temperate mesotrophic lakes (Fig. 2). Diatoms (Phytoplankton 1) strongly dominated the community in biomass. Changes in community SfCA occurred some 4–7 d in advance, even on short-term changes in ascendency (Fig. 3), the latter being synchronous with changes in total community biomass.

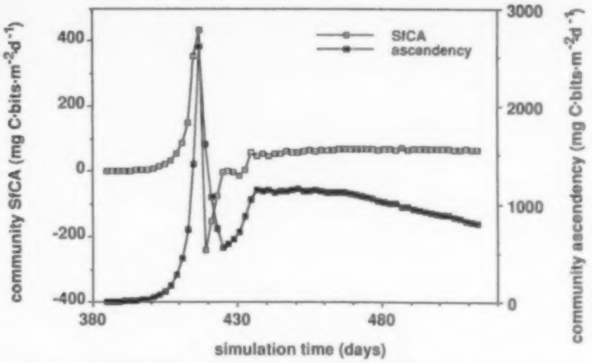

FIG. 3. Dynamics of community SfCA and of community ascendency over one season of growth in a simulation run.

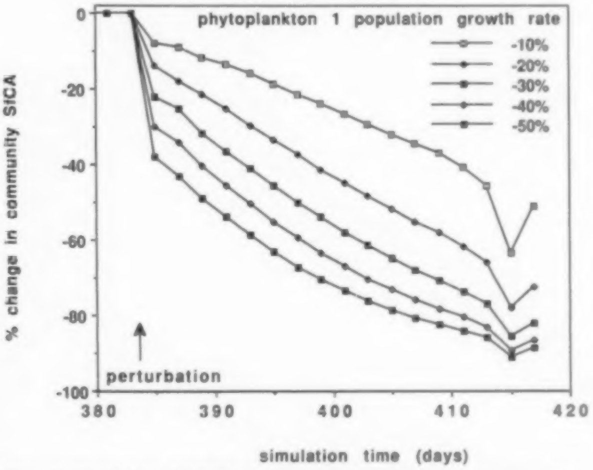

FIG. 4. Short-term inhibition of community SfCA as a response to a decrease in population growth rate of diatoms (Phytoplankton 1). In this simulation, extinction of the diatom population was not allowed to occur.

We simulated perturbations of population parameters (e.g. growth rate, grazing rate, and respiration rate) occurring at the beginning of the spring bloom and continuing throughout the simulation run. The model was run for one simulation year to obtain a steady state. Effects of perturbations on populations, measured as the relative decrease in community SfCA, were most evident in the case of disturbance on the phytoplankton, especially Phytoplankton 1, as this population was largely dominant in biomass during the spring bloom. A prolonged reduction of 10% in population growth rate of Phytoplankton 1 caused a decrease in community SfCA of 45% (from 152 to 83 mg $C\cdot bits\cdot m^{-2}\cdot d^{-1}$), and a reduction of 50% caused a decrease of 86% (from 152 to 21 mg $C\cdot bits\cdot m^{-2}\cdot d^{-1}$) within 28 d (Fig. 4). In another simulation, Phytoplankton 1 was assumed to go extinct when its production reached a critical low. The consequent alteration of community structure caused a further, steeper drop in SfCA (Fig. 5).

Thus, SfCA gave a sensitive and fast short-term response to changes in flow magnitudes and in topology. Differences in SfCA between "treatments," occurring after 30 d since perturbation, were difficult to interpret whereas small, consistent differences in ascendency remained observable over the scale of months or years. Other long-term changes in the community included accumulation of unused nutrients.

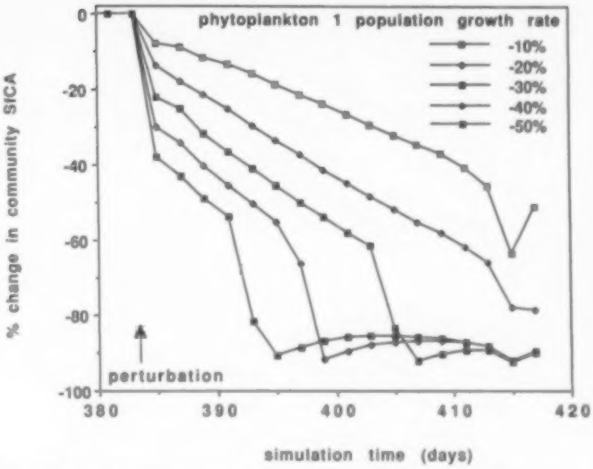

FIG. 5. Short-term inhibition of community SfCA as a response to a decrease in population growth rate of diatoms (Phytoplankton 1). In this simulation, the diatom population was assumed to go extinct as it dropped to a certain level.

In the simulation experiment described above (Fig. 4; no extinction), we compared the effect of the perturbation on the community and on Phytoplankton 1. To do this, we calculated the contribution of the Phytoplankton 1 population to the community SfCA by recalculating SfCA with only the flows involving this population. In spite of the strong dominance by Phytoplankton 1 in the community, changes in this population could not fully account for changes in the community (Fig. 6). This can be traced to an altered distribution of resources within the community in presence of a reduced Phytoplankton 1 population. Other phytoplankton populations took advantage of the reduced competition by diatoms and made up in part for the reduced community SfCA. This illustrates that community effects may be different from effects on a single species. In fact, with increasing disruption of Phytoplankton 1 growth, the relative drop in the SfCA contribution of this population was increasingly different from the relative drop in community SfCA (Fig. 6b). Yet other populations could not fully compensate for the decrease in community SfCA caused by the decline of the diatoms. This is shown by the decrease, with time, of the ratio between the two (Fig. 6c).

Implementation of the Approach

Implementation of this approach will be confronted with several difficult, but manageable, problems. Taking again the case of communities for illustration, these are the conceptual aggregation of organisms into community compartments, the selection of reference communities, the limitations inherent in the measurement of flows, size scale of experiments, and the spatio-temporal resolution of the measurements. We shall now briefly comment on each of these problems.

Aggregation of organisms of a community into compartments has traditionally been made according to taxonomy and to general trophic categories. A change in flow diversity is a good indicator of ecosystem stress (E. P. Odum 1985; Ulanowicz 1986). For these reasons, the use of ataxonomic aggre-

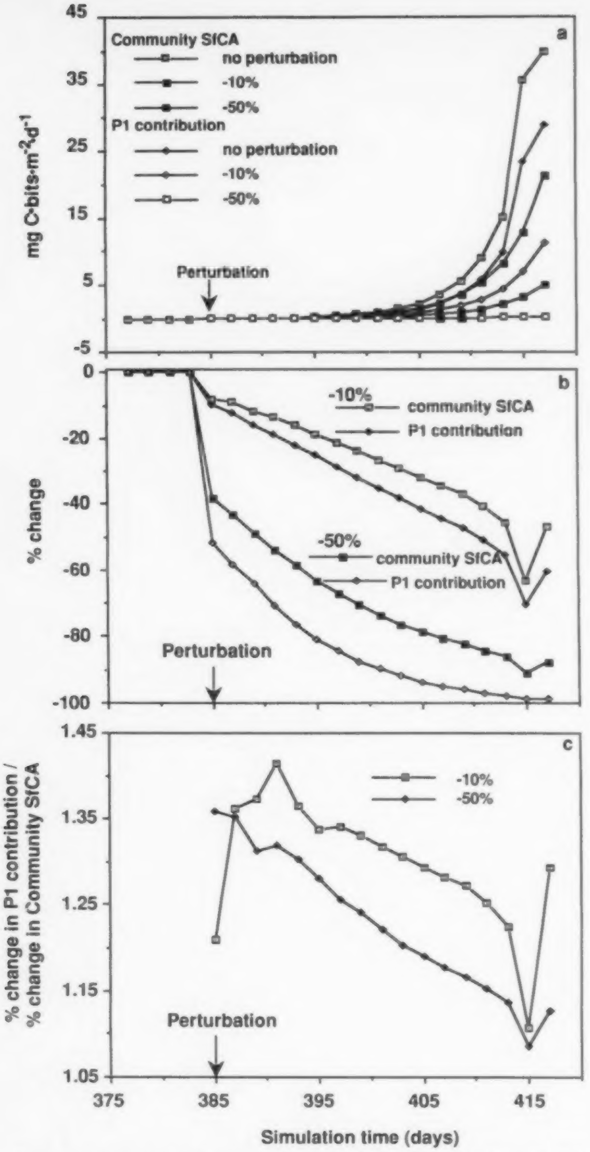

FIG. 6. Comparison of community SfCA and Phytoplankton 1 (P1) contribution to community SfCA. Simulations included, respectively, no perturbation, an inhibition of 10%, and an inhibition of 50% of the growth rate of Phytoplankton 1. (a) Absolute changes; (b) relative changes; (c) ratio of the relative changes.

gations has been advocated (Cummins 1974; Steel and Frost 1977; Odum and Biever 1984; Ulanowicz and Platt 1985; Moore and Hunt 1988). Such aggregations better reflect the diversity of flows, and should help achieve simplication and reduction in dimension of models in ways that reflect the structural and functional organization of the ecosystem (Levin et al. 1984). Still, the measurement of ascendency (and hence of SfCA) is sensitive to aggregation (Wulff et al. 1989). The aggregation that maximizes the amount of information preserved during the process of aggregation is preferrable (Hirata and Ulanowicz 1985). Since the implementation of community flow studies is only in its initial stage, now would be a good time to begin defining which mode and degree of aggregation is desirable for each type of community, so as to maximize our ability at identifying effects of stressors on community structure and function and of making comparisons across studies. The use of a standardized approach, a likely course for management of communities (Vouk et al. 1987), should then be possible. Model building should, in fact, always precede experimental work, so as to guide the design of studies (Denman et al. 1985). Most ecosystems possess distinct abiotic and biotic characteristics that dominate and that can be used to characterize them for this purpose. Perhaps the identification of only a few such factors (e.g. indicator or controlling species or processes) may be sufficient to define categories with respect to the effect of stressors (Levin et al. 1984; Rapport et al. 1985).

The selection of reference ecosystems to assess the similarity of reference and treatment sites before experimental manipulation is essential and may require several years of monitoring before experimentation. However, there is already much relevant information in the literature and in data files, and even though it may not include flows, it will be of much help in identifying suitable sites.

Identifying the essential compartments and quantitatively documenting flow dynamics may require the design of large-scale experiments, involving complex and expensive methods. Denman et al. (1985) suggested that direct measurement of flows would not require the design and construction of entirely new methods or instrumentation, but rather the development of existing instrumentation and methods into new forms. The problem of sampling grows exponentially with the size of the ecosystem considered. But that is not to say that portions of ecosystem cannot be interpreted meaningfully, and with reasonable funding (Emery and Mattson 1986). Moreover, as noted above, the knowledge gained from whole-ecosystem or mesocosm studies should help design assessment tools such as microcosm assays.

Natural variability, a fundamental property of biological systems, is a major limitation on the measurement of dynamic parameters such as trophic flows and on the predictive ability of ecosystem simulation models. This problem makes it necessary to use reasonably high sample sizes. Moreover, the spatio-temporal scale of the individual flow measurements will need to reflect the scale of the turnover rate of the various processes occurring within the ecosystem examined. For example, the amplitude and duration of a response to a stress may depend not only on toxicant input, but also on the intrinsic state of the mechanisms "transducing" the input into a community effect. The time dependency of the stress intensity and that of the response depend on different properties of the community. Thus, a description of stress effect will have to take into account the time-dependent processes involved in the expression of the response. A change in community SfCA may be surmised to be observable over a scale of days or weeks, and herein lies its appeal as an indicator of stress. In contrast, ecosystem functions such as ascendency likely show changes over a longer time (months or years).

Discussion

Theoretical arguments and simulation results suggest that SfCA may serve as a short-term descriptor of stress effects whereas ascendency appears to be the more reliable descriptor in the longer run. For example, a perturbation that affects the ascendency of a community for months or years may be detected by a change in SfCA within days or weeks. SfCA uses the flow network perspective, which provides a unified approach for the description of stress effects on all levels of biological organization, and allows for interlevel comparisons.

This approach allows describing flows of trophic commodities such as energy or nutrients, or flows of information. It may be extended to multicommodity networks (Costanza and Neill 1981) and to networks that are resolved along the space and time dimensions (Pahl-Wostl 1990, and in prep.). SfCA should apply to all kinds of biological networks, whatever their functional characteristics. For instance, its application to communities may be appropriate regardless of which trophic process dominates. In contrast, other descriptors of stress effects may have a more limited range of applications. For example, production quotient (Frutiger 1985) would probably meaningfully apply only to communities that are strongly consumer regulated.

In vivo assessment of SfCA can be backed up by computer simulations and thus be predictive as to interlevel repercussions and long-term effects. However, the temporal dynamics of ascendency are not yet completely understood, particularly as to the respective significance of short- and long-term variations therein. Lags in response, generated by the storage of reserves within populations, should reduce the close tracking of SfCA by ascendency, and hence compound the value of SfCA as a short-term descriptor. Although such lag effects may be relatively small in a community dominated by phytoplankton, we hypothesize that they may be significant in other communities. For this reason, we surmise that a most interesting line of research lies in the relationships between changes in SfCA, on one hand, and long-term changes in ascendency, on the other hand.

In addition, the use of SfCA may allow testing other hypotheses. Exploited ecosystems offer opportunities for hypothesis testing in the classical sense, i.e. through the observation of changes in ecosystem properties in response to perturbation (Leggett et al. 1985). For example, we may wish to ask whether SfCA is affected more strongly by a generic toxicant (with smaller effects on several populations), or a specific one (with larger effects on only one or a few populations), whether there are differences in response between communities in that respect, and whether species or processes may be ranked with respect to their influence on SfCA.

We have sought confirmation of the ecological relevance of this approach by measuring, in aquatic microcosms, short-term changes in carbon flows and in SfCA, and by comparing those changes between undisturbed microcosms and microcosms treated with a toxicant. Compounded and amplified effects of perturbations on populations, measured as a drop in SfCA of the community, were observed within 2–3 wk (Genoni 1990).

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References

BAYNE, B. L., D. L. HOLLAND, M. N. MOORE, D. M. LOWE, AND J. WIDDOWS. 1978. Further studies on the effect of stress in the adult on the eggs of *Mytilus edulis*. J. Mar. Biol. Assoc. U.K. 58: 825–841.

CALOW, P. 1989. Physiological ecotoxicology: theory, practice and application, p. 23–35. *In* Proceedings of the First European Conference on Ecotoxicology, Copenhagen, Denmark.

COSTANZA, R., AND C. NEILL. 1981. The energy embodied in the products of ecological systems: a linear programming approach, p. 661–670. *In* W. J. Mitsch and J. M. Klopatek [ed.] Energy and ecological modelling. Elsevier, Amsterdam, The Netherlands.

CUMMINS, K. W. 1974. Structure and function of stream ecosystems. Bioscience 24: 631–641.

DENMAN, K., W. CALDER, C. DAVIS, S. DEMERS, M. ESTRADA, M. LEWIS, D. SMITH, P. WANGERSKY, C. YENTSCH, AND A. ZOTIN. 1985. Technological developments to implement theory into biological oceanography. Can. Bull. Fish. Aquat. Sci. 213: 254–258.

EMERY, R. M., AND G. G. MATTSON. 1986. Systems ecology and environmental law: do they speak the same language?, p. 25–41. *In* T. M. Poston and R. Purdy [ed.] Aquatic toxicology and environmental fate. Vol. 9. ASTM Spec. Tech. Publ. 921. ASTM, Philadelphia, PA.

FRUTIGER, A. 1985. The production quotient PQ: a new approach for quality determination of slightly to moderately polluted running waters. Arch. Hydrobiol. 104: 513–526.

GENONI, G. P. 1990. Effect of a toxicant on scope for change in ascendency, a novel descriptor of community stress, in microcosms. Fifth International Congress of Ecology, Japan. p. 478. (Abstr.)

GENONI, G. P., AND C. PAHL-WOSTL. 1990. Scope for change in ascendency, a new concept in community ecotoxicology for environmental management. *In* O. Ravera [ed.] Terrestrial and aquatic ecosystems: perturbation and recovery. Proceedings of the Fifth European Ecology Symposium. Ellis Horwood Ltd., Chichester.

GILFILLAN, E. S. 1980. The use of scope-for-growth measurements in monitoring petroleum pollution. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 179: 71–75.

HIRATA, H., AND R. E. ULANOWICZ. 1984. Information theoretical analysis of ecological systems. Int. J. Syst. Sci. 15: 261–270.

1985. Information theoretical analysis of the aggregation and hierarchical structure of ecological networks. J. Theor. Biol. 116: 321–341.

HOCHACHKA, P. W., AND G. N. SOMERO. 1984. Design of cellular metabolism, chap. 2. *In* Biochemical adaptation. Princeton University Press, Princeton, NJ.

KOOIJMAN, S. A. L. M., AND J. A. J. METZ. 1984. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. Ecotoxicol. Environ. Safety 8: 254–274.

LEGGETT, W. C., W. R. BOYNTON, V. F. GALLUCCI, L. JOHNSON, R. MARGALEF, J. MCGLADE, D. MIKULECKY, R. ROSEN, R. O. ULLOA, AND J. WATSON. 1985. Hypothesis testing and sampling design in exploited ecosystems. Can. Bull. Fish. Aquat. Sci. 213: 237–240.

LEVIN, S. A., K. D. KIMBALL, W. H. MCDOWELL, AND S. F. KIMBALL. 1984. New perspectives in ecotoxicology. Environ. Manage. 8: 375–442.

MARGALEF, R. 1981. Stress in ecosystems: a future approach, p. 281–289. *In* G. W. Barrett and R. Rosenberg [ed.] Stress on natural ecosystems. Wiley, New York, NY.

MOORE, J. C., AND H. W. HUNT. 1988. Resource compartmentation and the stability of real ecosystems. Nature (Lond.) 333: 261–263.

ODUM, E. P. 1969. The strategy of ecosystem development. Science (Wash., DC) 164: 262–270.

1981. The effects of stress on the trajectory of ecological succession, p. 43–48. *In* G. W. Barrett and R. Rosenberg [ed.] Stress effects on natural ecosystems. Wiley, New York, NY.

1985. Trends expected in stressed ecosystems. Bioscience 35: 419–422.

ODUM, E. P., AND L. J. BIEVER. 1984. Resource quality, mutualism, and the energy partitioning in food chains. Am. Nat. 124: 360–376.

ODUM, H. T. 1985. Systems ecology: an introduction. Wiley, New York, NY.

PAHL-WOSTL, C. 1990. Temporal organization: a new perspective on the ecological network. Oikos 58: 293–305.

RAPPORT, D. J., H. A. REGIER, AND T. C. HUTCHINSON. 1985. Ecosystem behavior under stress. Am. Nat. 125: 617–640.

SCAVIA, D. 1980. An ecological model of lake Ontario. Ecol. Model. 8: 49–78.

SCHINDLER, D. W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl.1): 6–25.

STEELE, J. H., AND B. W. FROST. 1977. The structure of plankton communities. Philos. Trans. R. Soc. Lond. B 280: 485–534.

ULANOWICZ, R. E. 1986. Growth and development. Ecosystem phenomenology. Springer-Verlag, New York, NY. 203 p.

ULANOWICZ, R. E., AND W. M. KEMP. 1979. Toward canonical trophic aggregations. Am. Nat. 114: 871–883.

ULANOWICZ, R. E., AND T. PLATT [ED.] 1985. Ecosystem theory for biological oceanography. Can. Bull. Fish. Aquat. Sci. 213: 260 p.

VOUK, V. B., G. C. BUTLER, A. C. UPTON, D. V. PARK, AND S. C. ASHER. 1987. Methods for assessing the effects of mixtures of chemicals. 1. Introduction, general conclusions and recommendations. SCOPE 30, SGOMSEC 3, p. 3–13. Wiley, Chichester.

WARREN, C. E., AND G. E. DAVIS. 1967. Laboratory studies on the feeding bioenergetics and growth of fish, p. 201–214. *In* S. D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell Scientific Publications, Oxford.

WIENS, J. A. 1984. Resource systems, populations, and communities, p. 397–436. *In* P. W. Price, C. N. Slobodchikoff, and W. S. Gaud [ed.] A new ecology: novel approaches to interactive systems. Wiley, New York, NY.

WINBERG, C. G. 1960. Rate of metabolism and food requirements of fish. Fish. Res. Board Can. Transl. Ser. 194: 202.

WIRTH, N., J. GUTKNECHT, W. HEIZ, H. SCHAER, H. SEILER, AND C VETTERLI. 1988. MacMETH, User manual. 2nd ed. Wolf T. [ed.] Institut fuer Informatik, ETH Zuerich, Zuerich, Switzerland. 100 p.

WULFF, F., J. G. FIELD, AND K. H. MANN. 1989. Network analysis in marine ecology. Methods and applications. Coastal and estuarine studies. No. 32. Springer-Verlag, Berlin. 284 p.

Appendix: Derivation of Scope for Change in Ascendency

Ascendency has the form (Ulanowicz 1986)

$$A = \sum_{j=0}^{n} \sum_{i=1}^{n+2} T_{ji} \, log \ldots \frac{T_{ji}T}{T_{j\cdot}T_{\cdot i}}$$

where T is the throughput, T_{ji} are flows from compartments j to compartments i, $T_{j\cdot}$ are total compartment outputs, and $T_{\cdot i}$ are total compartment inputs. By separating the input and output terms, this can be written as

$$\begin{aligned} A &= \sum_{j=0}^{n} \sum_{i=1}^{n+2} \frac{T_{ji}}{2} \left[\log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{\cdot i}{}^{2}} + \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{j\cdot}{}^{2}} \right] \\ &= \sum_{j=0}^{n} \sum_{i=1}^{n+2} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{\cdot i}{}^{2}} \\ &\qquad + \sum_{j=0}^{n} \sum_{i=1}^{n+2} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{j\cdot}{}^{2}} = A_{\mathrm{in}} + A_{\mathrm{out}} \end{aligned}$$

where A_{in} is the ascendency of inputs and A_{out} is the ascendency of outputs. For expressing SfCA, we take into account only internal compartments. Let $\tilde{A}_{\mathrm{in}}$ and $\tilde{A}_{\mathrm{out}}$ refer to these internal compartments only. Then:

$$\begin{aligned} A &= \sum_{j=0}^{n} \sum_{i=1}^{n} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{\cdot i}{}^{2}} + \sum_{j=0}^{n} \sum_{i=n+1}^{n+2} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{\cdot i}{}^{2}} \\ &\quad + \sum_{j=0}^{n} \sum_{i=1}^{n+2} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{j\cdot}{}^{2}} + \sum_{i=1}^{n+2} \frac{\mathrm{T}_{0i}}{2} \log \frac{\mathrm{T}_{0i}\mathrm{T}}{\mathrm{T}_{0\cdot}{}^{2}} \end{aligned}$$

where the first term is $\tilde{A}_{\mathrm{in}}$ and the third term is $\tilde{A}_{\mathrm{out}}$. Let SfCA be the difference between $\tilde{A}_{\mathrm{in}}$ and $\tilde{A}_{\mathrm{out}}$:

$$\mathrm{SfCA} = \sum_{j=0}^{n} \sum_{i=1}^{n} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{\cdot i}{}^{2}} - \sum_{j=0}^{n} \sum_{i=1}^{n+2} \frac{\mathrm{T}_{ji}}{2} \log \frac{\mathrm{T}_{ji}\mathrm{T}}{\mathrm{T}_{j\cdot}{}^{2}} .$$

An Assessment of Chironomidae as Quantitative Indicators of Past Climatic Change

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Walker, I. R., J. P. Smol, D. R. Engstrom, and H. J. B. Birks. 1991. An assessment of Chironomidae as quantitative indicators of past climatic change. Can. J. Fish. Aquat. Sci. 48: 975–987.

The distribution among Labrador (Canada) lakes of remains from 21 chironomid taxa was analysed by means of canonical correspondence analysis (CCA). Tests with partial CCA of three hypotheses revealed that the distributions of chironomid taxa were significantly correlated with summer surface-water temperature and maximum lake depth, but not sediment organic content. In addition, an exploratory CCA revealed possible relationships of chironomid fauna with residual longitude and Fe concentration. A weighted-averaging-regression/calibration model of the chironomid–temperature relationship (derived from a temperature-constrained CCA) allowed the summer surface-water temperature of lakes to be inferred from chironomid remains. Accurate quantitative reconstructions of late glacial and Holocene temperature variations should be possible from fossil Chironomidae.

On a analysé par la méthode canonique des correspondances la distribution dans les lacs du Labrador (Canada) des débris de chironomides de 21 taxons. La vérification par analyse canonique partielle de trois hypothèses a révélé que la distribution des taxons de chironomides était en corrélation significative avec la température de l'eau de surface en été et la profondeur maximale du lac, mais non avec la teneur en matières organiques des sédiments. En outre, une analyse canonique exploratoire a révélé l'existence possible de relations entre la faune de chironomides et la longitude résiduelle ainsi que la concentration de Fe. Un modèle d'étalonnage avec régression, calcul de moyenne et pondération de la relation chironomide–température (conçu à partir d'une analyse canonique à température imposée) a permis de déterminer la température de l'eau de surface des lacs en été d'après les débris de chironomides qu'on a trouvés. Il devrait être possible de produire des reconstitutions quantitatives exactes des variations de température de la fin de l'époque glaciaire et de l'holocène d'après les fossiles de chironomides.

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The distributions of aquatic midges among Canadian climatic regions suggest that temperature limits the present range of many taxa, particularly in arctic and alpine environments (Walker and Mathewes 1989a). Despite such evidence, the merits of Chironomidae, and other aquatic invertebrates, as palaeoclimatic indicators have been vigorously debated (Hann and Warner 1987; Walker 1991; Walker and Mathewes 1987a, 1987b, 1989a, 1989b, 1989c; Warner and Hann 1987; Warwick 1989). Warner and Hann (1987) and Warwick (1989) have emphasized the importance of turbidity, substrate composition, and sedimentation rates as regulators of species distributions, especially the distributions of those taxa common in late glacial deposits. As determinants of late glacial/early Holocene chironomid succession, Walker and Mathewes (1987b, 1989c) argued that these relationships are less important than climatic factors. Many taxa common during late glacial time declined greatly in abundance, or completely disappeared from many temperate lakes ca. 10 000 yr B.P., during a period of rapid climatic amelioration.

Distributions of many aquatic organisms among lakes can be most profitably studied through analyses of their remains in surficial lake sediments (e.g. Boubée 1983; DeCosta 1964; Douglas and Smol 1987; Meriläinen 1967; Synerholm 1979; Walker and Mathewes 1989a; Whiteside 1970). Factors influencing biological composition can then be identified from multivariate analysis. Canonical correspondence analysis (CCA), an ordination procedure recently developed by ter Braak (1986, 1987a, 1989), appears to provide the best available means for analysing such data (e.g. Dixit et al. 1989; Stevenson et al. 1989). This procedure has previously been used to examine factors governing the distribution of living organisms, as well as fossils. For example, using CCA, Johnson and Wiederholm (1989) identified significant relationships between profundal macroinvertebrate fauna and water temperature, depth, pH, total P, SO_4^{2-}, $Si(OH)_4$, and phytoplankton biovolume. Other applications of CCA appearing in the freshwater ecological literature include studies by Chessel et al. (1987) and Fängström and Willén (1987). Although most often employed for exploratory data analysis, CCA also provides means for hypothesis testing (e.g. de Bakker 1989).

The organism–environment relationships revealed by CCA can later be used for direct quantitative reconstructions of past environmental conditions (e.g. Dixit et al. 1989; Stevenson et al. 1989; ter Braak 1987a; ter Braak and van Dam 1989). The weighted-averaging regression and calibration procedures described by ter Braak (1987b) and ter Braak and Looman (1987) allow direct quantitative reconstructions of several environmental variables independently from the same fossil data (e.g. Birks et al. 1990; Kingston and Birks 1990).

To test hypotheses arising from Walker and Mathewes' (1989a), Warner and Hann's (1987), and Warwick's (1989) arguments, we have examined the distribution of chironomid remains among lakes in Labrador and adjacent Quebec. A priori, three null hypotheses were identified for our analysis: the relative abundance of fossil chironomid taxa in surficial sediments is not correlated with (1) summer surface-water temperature, (2) maximum lake depth, or (3) organic content of the sample. Unfortunately, sediment accumulation rates were not measured for the lakes included in our survey. Thus, a rigorous test of Warwick's (1980, 1989) hypothesis (i.e. that chironomid faunistic composition is influenced by the rate of mineral sediment accumulation) is not possible. In those arctic lakes that do not receive glacial meltwater, sediment accumulation rates are usually much lower than natural sediment accumulation rates in lakes of subarctic or temperate regions (Moser 1988; Webb and Webb 1988).

Although correlations between chironomid faunistic composition and P, oxygen availability, chlorophyll *a*, humic content, Fe, pH, and food quantity and quality have been suggested (e.g. Brundin 1951; Mossberg and Nyberg 1979; Rasmussen and Lindegaard 1988; Sæther 1979), we had not constructed hypotheses pertaining to these variables a priori. No measures of food quantity, food quality, chlorophyll *a*, or oxygen concentration were available.

The merits of Chironomidae as palaeoclimatic indicators can be even more rigorously tested through attempts to infer the surface-water temperature of present-day lakes from a weighted-averaging-regression/calibration model of the Labrador chironomid–temperature relationship. We use a Labrador model to infer temperatures for British Columbia lakes spanning an altitudinal gradient. Although summer surface-water temperatures are not available for all of these lakes, surface-water temperatures in British Columbia are obviously related to altitude. Thus, we anticipated a significant correlation between inferred surface-water temperatures and altitude in British Columbia.

Study Area

The study lakes are broadly scattered throughout Labrador and adjacent Quebec, spanning low-arctic tundra, forest–tundra, subarctic lichen–woodland, and boreal spruce–fir forest vegetation zones (Fig. 1). In southeastern Labrador, extensive areas of muskeg and peatland have developed. The vegetation at the three southernmost sites was classified by Rowe (1972) as coastal tundra, but this coastal belt is distinguished by dense thickets of spruce and fir, locally known as "tuckamore." A more detailed description of vegetation at the study sites was provided by Lamb (1984).

The lakes of Labrador are dilute, and pH ranges from 5.0 to 7.5, reflecting the cool, moist climate and the granitic nature of the underlying bedrock. Lakes of southern Labrador tend to have higher ionic concentrations, as well as higher total P. The shallow lakes of southeastern Labrador, especially those situated in peatlands, are often highly coloured and have high Fe concentrations.

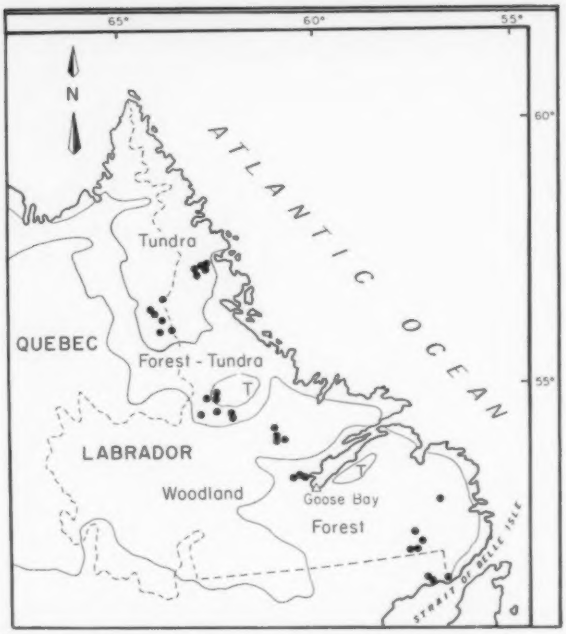

FIG. 1. Map illustrating locations for those Labrador lakes where surficial sediments were analysed for midge remains (adapted from Engstrom 1987).

Methods

Sampling and Subsampling

The small samples of surficial lake sediments that we have examined had originally been collected to assess the distribution of pollen (Lamb 1984) and diatoms in lake sediments near the tree line and across the subarctic forest zone. The physical and chemical characteristics of these lakes were also documented to obtain data pertaining to the quality of surface waters (Clair et al. 1982) and to develop humus budgets for Labrador lakes (Engstrom 1987).

Sediment sampling procedures have been described by Lamb (1984). Seventy-four lakes in Labrador and adjacent Quebec were sampled via floatplane during July–August 1979. Sediments were collected near the point of maximum depth of lakes having no major inflowing stream. Depth was determined by means of an electronic sounder mounted on the aircraft's pontoon. The uppermost 3–5 cm of sediment was collected by means of a free-fall, modified Hongve sampler (Wright 1990).

Following the pollen and diatom analyses, sufficient sediment remained in many of the samples for the analysis of chironomid remains. Samples for chironomid analysis were selected from this original set so as to represent a large gradient in latitude, maximum depth, and sediment organic content. Samples which appeared to contain an insufficient volume of sediment for analysis were avoided. In total we examined sediments from 33 of Lamb's (1984) lakes (i.e. lakes 1, 2, 3, 10, 14, 16, 17, 20, 22, 23, 24, 28, 29, 32, 34, 35, 36, 41, 42, 43, 45, 46, 48, 49, 50, 52, 53, 54, 56, 58, 61, 64, and 68). The coordinates for these lakes are provided in Lamb (1984).

In samples from seven lakes (lakes 23, 24, 32, 48, 49, 52, and 68), fewer than 50 chironomid remains could be found.

Chironomid Analysis

For chironomid analysis, 1–2 mL of sediment (where available) was deflocculated in warm 5% KOH and sieved on a 95-µm Nitex® mesh. The sediment retained on the sieve was washed into a beaker and later examined in a Bogorov counting tray (Gannon 1971) at a magnification of 50× for chironomid remains, and other fossils. All head capsules of Chironomidae and Ceratopogonidae, mandibles of *Chaoborus*, and remains of Simuliidae were mounted on slides in Permount®. Chironomid remains were identified with reference to Oliver and Roussel (1983), Walker (1988), and Wiederholm (1983). Dr. A. J. Uutala (Biology Department, Queen's University) and the descriptions of Cook (1956), Sæther (1970), and Uutala (1990) were consulted for identification of *Chaoborus*. Chironomids were mostly identifiable to the generic level. In some instances, however, larger taxonomic groupings were necessary (e.g. *Corynoneura/Thienemanniella*, *Cricotopus/Orthocladius*, *Tanytarsus* s. lat). Most characters used for identification of these fossils were described by Walker (1988).

Chironomid head capsules that included the complete mentum were counted as one head capsule. Head capsule fragments that included precisely half the mentum (common among Orthocladiinae fossils) were counted as half a head capsule. Many head capsules had split such that the mentum had broken into two unequal halves. Usually only the more complete half was identifiable, and this portion was counted as one head capsule. In some instances, however (e.g. *Cladopelma* and *Pseudodiamesa*), fragments including less than half the mentum were identifiable on the basis of extreme lateral mental teeth. In such instances the fragments including less than half the mentum and greater than half the mentum were both counted as half a head capsule.

Measurement of Environmental Variables

Water chemistry determinations were made from epilimnetic samples collected 0.5 m below the lake surface in 1979. Specific conductance, pH, apparent colour, and alkalinity (by gran titration) were measured in the field, and additional samples were retained for laboratory analysis of cations (by DC plasma spectrometry), anions (by ion chromatography), and total P (by the ascorbic acid method; American Public Health Association 1980). Lake surface areas were measured on 1:50 000 topographic maps, and predominant catchment vegetation was determined from 1:17 000 aerial photographs. Sediment organic content was measured by loss on ignition at 550°C.

Data Analysis

CCA was performed using the program CANOCO, version 3.0 (ter Braak 1989). Input for the program included percentage taxon data for all lakes where at least 50 chironomid head capsules were recovered. Taxa that did not constitute $\geq 2\%$ of the fauna in at least two Labrador lakes were excluded.

Hypothesis Testing

To assess directly the hypotheses outlined in this paper's introduction, a series of partial, constrained, CCAs was performed. Partial CCA allows the effects of covariables to be partialled out during statistical analysis. The theory of partial CCA was described by ter Braak (1987c). The significance of each of three variables (summer surface-water temperature, maximum lake depth, and sediment organic content) as determinants of chironomid fauna was assessed against all possible combinations of four covariables (summer surface-water temperature, maximum depth, sediment organic content, and number of identifiable head capsules), as indicated in Table 1. Transformation with log $(x + 1)$ was necessary to alleviate the skewed nature of the data for maximum depth and number of identifiable head capsules. The significance of each variable was assessed by means of a Monte Carlo permutation test for significance of the first (constrained) ordination axis. In all tests, significance was assessed on the basis of $\alpha = 0.05$.

Exploratory Data Analysis

To identify other environmental variables that might exert an additional influence on the chironomid fauna, an exploratory CCA was also performed. The environmental data included 23 variables measured for each lake in 1979. These data included water chemistry (pH, alkalinity, specific conductance, Ca, Mg, Na, K, Cl, SO_4, Fe, total P, and total Si), Secchi depth, water colour, lake area and elevation, latitude, and three vegetation zones (arctic tundra, subarctic woodland (includes both forest–tundra and lichen–woodland), and boreal forest (including some lakes in peatlands), each entered as binary codes), as well as sediment organic content, maximum depth, and summer surface-water temperature.

Many of the environmental variables displayed strongly skewed distributions (i.e. maximum depth, colour, Secchi depth, specific conductance, alkalinity, total P, total Si, Ca, Mg, Na, K, Fe, SO_4, and Cl). To eliminate, or at least to reduce, this effect, each of these variables was transformed (log $(x + 1)$). Two lakes (2 and 10) with unusual water chemistry were identified as outliers and were excluded during this exploratory analysis.

Because the lakes were visited along a northwest-trending sampling transect, latitude and longitude were highly correlated. This autocorrelation was removed by analysing only the residual longitude (e_i) that could not be predicted from the mean trajectory of the transect. (The position of lake i on the transect is described by the regression equation $Y_i = 1.327\ X_i - 11.112 + e_i$, where Y_i and X_i are, respectively, the longitude and latitude of lake i.)

Variables to be included in the ordination were chosen with the forward selection option of CANOCO, version 3.0 (ter Braak 1989). Forward selection in CCA is analogous to the selection process employed in stepwise multiple regression. The variables are selected for the CCA such that each will account for the greatest possible proportion of the remaining variance. Only variables that explained a significant ($P < 0.05$) additional proportion of total variance were included. Significance was assessed on the basis of the Monte Carlo permutation test available with forward selection in CANOCO, version 3.0 (ter Braak 1989). Forward selection also determined the order in which variables were entered into the analysis.

Temperature Inference

An empirical weighted-averaging-regression/calibration model of the chironomid–temperature relationship was later developed from a temperature-constrained CCA. The procedure used in developing this model has previously been used to reconstruct pH from fossil diatoms and chrysophytes (e.g.

Dixit et al. 1989; Stevenson et al. 1989; ter Braak and van Dam 1989). The weighted-averaging model was obtained, as described for the preceding analysis, but lakes 2 and 10 were included, and summer surface-water temperature was the only environmental variable included in this analysis. The regression equation derived from the output of this constrained analysis is

$$(1) \quad \hat{T}_i = t + [S_t \cdot A_i / B]$$

where $\hat{T}_i$ is the predicted summer surface-water temperature for lake i, t is the mean summer surface-water temperature for all lakes used in developing the weighted-averaging model, S_t is the standard deviation of temperature for the same lakes, A_i is the CCA sample score for lake i, and B is the canonical coefficient for temperature.

This weighted-averaging model constitutes a transfer function that may be used to predict summer surface-water temperatures from fossil data. Using this model, calibration of British Columbia data (chironomid remains from surficial lake sediments: Walker and Mathewes 1989a) was achieved by including British Columbia lakes as passive samples in the analysis. This procedure follows the regression/calibration procedures described by ter Braak (1987a) and employed for pH inference by Stevenson et al. (1989). Predicted summer surface-water temperatures could then be calculated from Eq. 1. For comparative purposes, data for several other regions were also included as passive samples. No passive samples were entered that contained fewer than 50 head capsules.

Results

Chironomid remains were common in most Labrador sediments, but fossils of other Diptera were rare. Only two *Chaoborus* mandibles were found (lakes 2 and 29), both identified as *Chaoborus trivittatus*. One simulid mandible was found in sediment from lake 29. Ceratopogonidae were rare, being found only in lakes 14, 20, 41.

Hypothesis Testing

Tests of summer surface-water temperature and maximum lake depth via partial CCA revealed that these variables were significant correlates of the chironomid distributions, regardless of the covariables designated in each analysis (Table 1). In no instance was sediment organic content identified as a significant correlate. We conclude that summer surface-water temperature and maximum lake depth influence the fauna and that sediment organic content of core samples is not a useful predictor of fossil chironomid fauna. Although the number of head capsules recovered from each sample varied greatly, this had no significant influence upon these results.

Exploratory Data Analysis

With forward selection, CCA identified four environmental variables that explained significant proportions of the variance of the faunal weighted averages (Table 2): summer surface-water temperature ($P = 0.001$), residual longitude ($P = 0.001$), maximum depth ($P = 0.013$), and Fe ($P = 0.032$). Summer surface-water temperature represents 22% of the total variance explained by all environmental variables. With forward selection, residual longitude, maximum lake depth, and Fe explained an additional 11, 8, and 7%, respectively, of the variance explained by all environmental variables (Table 2).

TABLE 1. Results of partial CCA permutation tests for significance of axis 1 (Temp = summer surface-water temperature; Dpth = log (maximum depth + 1); Org = sediment organic content; NoHC = log (number of head capsules identified + 1)).

Variable	Covariables	Eigen-value 1	Eigen-value 2	Significance (*P*) of Monte Carlo permutation test
Temp	Dpth, Org, NoHC	0.102	0.136	0.01
	Dpth, Org	0.101	0.149	0.01
	Dpth, NoHC	0.102	0.149	0.01
	Org, NoHC	0.173	0.143	0.01
	Dpth	0.101	0.165	0.01
	Org	0.215	0.176	0.01
	NoHC	0.174	0.155	0.01
	None	0.221	0.193	0.01
Dpth	Temp, Org, NoHC	0.055	0.136	0.03
	Temp, Org	0.077	0.149	0.02
	Temp, NoHC	0.055	0.149	0.02
	Org, NoHC	0.126	0.136	0.01
	Temp	0.080	0.165	0.03
	Org	0.191	0.150	0.01
	NoHC	0.127	0.149	0.01
	None	0.200	0.165	0.01
Org	Dpth, Temp, NoHC	0.038	0.136	0.17
	Dpth, Temp	0.041	0.149	0.26
	Dpth, NoHC	0.037	0.136	0.28
	Temp, NoHC	0.038	0.143	0.26
	Dpth	0.041	0.150	0.16
	Temp	0.044	0.176	0.13
	NoHc	0.038	0.220	0.36
	None	0.051	0.295	0.16

TABLE 2. Variance potentially explained by each environmental variable before forward selection and variance explained with the addition of each environmental variable during forward selection.

Variable	Before	Added with selection
Temperature	0.20	0.20
Depth	0.18	0.07
Secchi depth	0.18	
Latitude	0.17	
Tundra	0.12	
Total *P*	0.12	
Colour	0.11	
Forest	0.11	
Fe	0.11	0.06
Res. longitude	0.10	0.10
Organic content	0.08	
SO_4	0.07	
Elevation	0.07	
Conductance	0.07	
Mg	0.07	
Ca	0.06	
Na	0.06	
Cl	0.06	
Alkalinity	0.05	
pH	0.05	
K	0.04	
Si	0.03	
Woodland	0.03	
Lake area	0.02	
Sum of variance	0.91	0.43

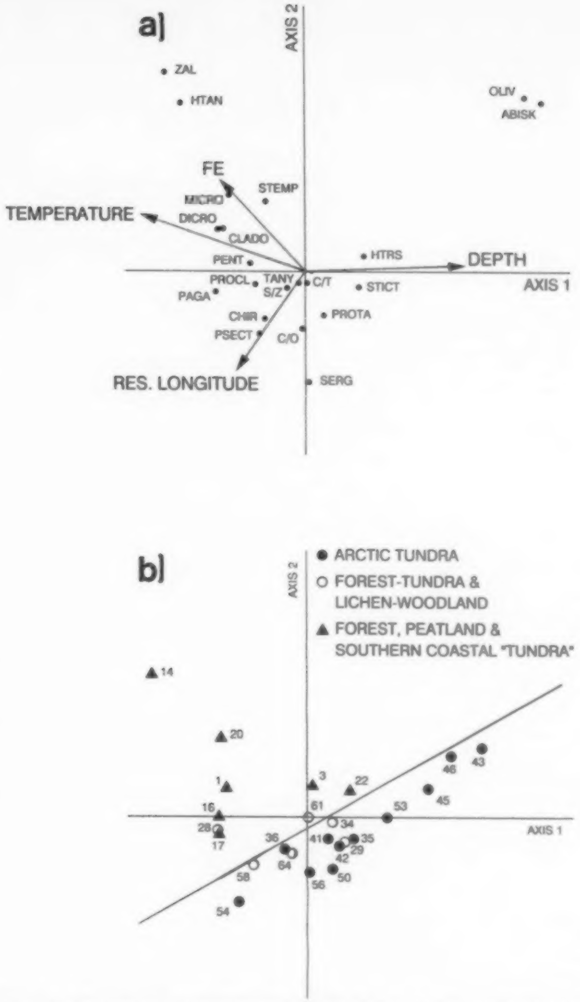

FIG. 2. Ordination of (a) chironomid taxa and environmental variables in Labrador, Canada, and (b) lakes. Abbreviations for chironomid taxa: ABISK, *Abiskomyia*; CHIR, *Chironomus*; C/O, *Cricotopus/Orthocladius*; C/T, *Corynoneura/Thienemanniella*; CLADO, *Cladopelma*; DICRO, *Dicrotendipes*; HTAN, *Heterotanytarsus*; HTRS, *Heterotrissocladius*; MICRO, *Microtendipes*; OLIV, *Oliveridia*; PAGA, *Pagastiella*; PENT, Tribe Pentaneurini; PROCL, *Procladius*; PROTA, *Protanypus*, PSECT, *Psectrocladius*; S/Z, *Stempellinella/Zavrelia*; SERG, *Sergentia*; STEMP, *Stempellina*; STICT, *Stictochironomus*; TANY, *Tanytarsus* s. lat; ZAL, *Zalutschia*. Lakes are numbered in accordance with Lamb (1984).

TABLE 3. Weighted correlation matrix (weight = sample total) relating taxon axes to measured environmental variables (= interset correlations of environmental variables with axes).

	Axis			
	1	2	3	4
Max. depth	0.77	0.04	−0.28	−0.31
Temperature	−0.79	0.37	−0.18	−0.06
Fe	−0.41	0.59	−0.01	0.42
Res. longitude	−0.34	−0.64	−0.35	0.22
Fraction of variance	0.37	0.22	0.06	0.08

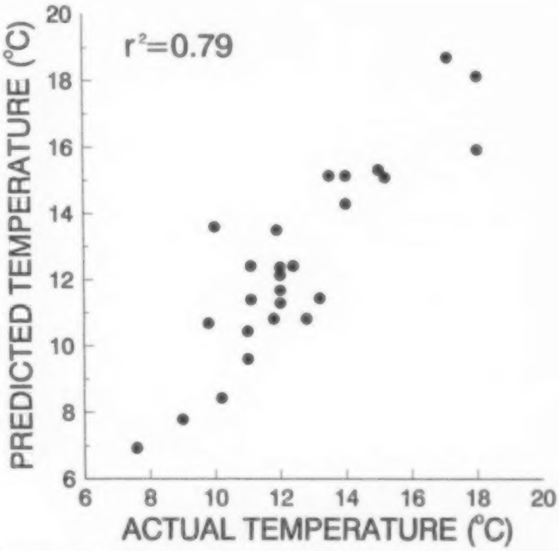

FIG. 3. Relationship between actual and chironomid-inferred summer surface-water temperatures for Labrador lakes.

Thus, these four variables account for 47% of all explainable variance of the weighted averages of the taxa.

CCA provides a combined ordination of the taxa, lakes, and environmental variables (Fig. 2). The first and second CCA axes, respectively, explained 26.1 and 10.1% of the variance explained by all (23) environmental variables. The ordination illustrates that summer surface-water temperature ($r = -0.79$) and maximum depth ($r = 0.77$) are strongly correlated with taxon axis 1 (Table 3). Fe ($r = 0.59$) and residual longitude ($r = -0.64$) are most strongly correlated with axis 2. *Abiskomyia*, *Oliveridia*, and *Heterotrissocladius* are associated with the coldest lakes (deep lakes in northernmost Labrador). *Paracladius*, *Parakiefferiella* sp. A, and *Pseudodiamesa* were also recorded from these low arctic lakes but were too rare to be included in this analysis. Characteristic taxa of warm, low-latitude lakes are mostly littoral inhabitants, including especially members of the tribe Chironomini (e.g. *Cladopelma*, *Dicrotendipes*, *Microtendipes*, and *Pagastiella*). The warmest lakes sampled were shallow, humic, Fe-rich lakes of southeastern Labrador where *Zalutschia*, *Heterotanytarsus*, and Ceratopogonidae were most common. The arrangement of taxa along axis 1 parallels that which would be expected from the altitudinal distribution of Chironomidae in British Columbia (Walker and Mathewes 1989a).

Ordination of the lakes on the first two axes reveals a good separation between lakes of the arctic tundra and those exposed to comparatively mild climates of southern Labrador (Fig. 2b). Lakes of subarctic lichen–woodland and forest–tundra sites occupy intermediate positions.

Temperature Inference

To use CCA for reconstructing past temperatures, the eigenvalue (λ_1) for the first (constrained) axis must be large relative to the eigenvalues for other axes (ter Braak 1987a). Since

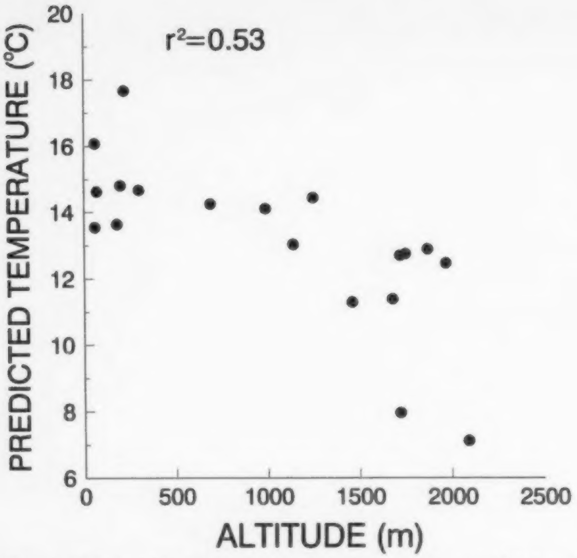

FIG. 4. Relationship between lake altitude and chironomid-inferred summer surface-water temperatures in southwestern British Columbia.

$\lambda_1/\lambda_2 = 1.15$ in our analysis, temperature is the dominant variable represented by axis 1. It should, therefore, be possible to obtain good temperature reconstructions from fossil chironomid data.

The weighted-averaging model of Labrador chironomid–temperature relationships yields predicted temperatures for Labrador lakes that closely approximate actual values (Fig. 3). The apparent root-mean-square-error for the temperature predictions is 1.32°C.

This model, however, is much more rigorously tested when applied to the British Columbia data. Predicted temperatures are significantly correlated with altitude in British Columbia (Fig. 4). The weighted-averaging model, developed from Labrador surface sample collections, tends to underestimate the temperature of low-elevation surface waters and may overestimate the temperature for several of the highest elevation lakes (including those receiving glacial meltwater). Unfortunately, actual temperature data are available for few of these British Columbia lakes. Where temperature data were obtained, most of the British Columbia lakes had thawed only a few days earlier. Such measurements are unlikely to be good estimates of maximum summer temperature.

Discussion

Temperature and Depth

The preceding analysis supports Walker and Mathewes' (1989a) suggestion that water temperature significantly influences chironomid faunistic composition and their belief that chironomids are useful palaeoclimatic indicators. Summer surface-water temperature is identified as explaining the greatest proportion of variance of the weighted averages of taxa. Surface temperatures would not be expected to influence greatly profundal fauna. However, many of the lakes sampled are shallow; even in deep lakes, remains of littoral invertebrates are transported far offshore prior to deposition and are well represented in deepwater sediments (Frey 1988).

TABLE 4. Weighted correlation matrix for environmental variables (following transformations) measured at the 24 lakes included in the exploratory CCA.

	ELEV	LAT	AREA	DEPTH	TEMP	SECC	TOT-P	COND	pH	ALK	Cl	SO_4	Na	K	Ca	Mg	Fe	Si	COLOR	FORST	WOODL	TUNDR	ORG	Res. LONG
LAT	0.67	1.00																						
AREA	−0.01	−0.07	1.00																					
DEPTH	−0.16	0.28	−0.03	1.00																				
TEMP	−0.42	−0.84	0.08	−0.59	1.00																			
SECC	0.24	0.59	−0.09	0.79	−0.69	1.00																		
TOT-P	−0.05	−0.56	0.16	−0.64	0.71	−0.68	1.00																	
COND	−0.58	−0.78	0.07	0.02	0.56	−0.37	0.23	1.00																
pH	0.22	0.46	−0.14	0.12	−0.31	0.32	−0.29	−0.42	1.00															
ALK	−0.24	−0.55	0.08	−0.08	0.41	−0.33	0.14	0.81	−0.34	1.00														
Cl	−0.82	−0.64	−0.01	0.25	0.28	−0.10	0.10	0.58	−0.13	0.20	1.00													
SO_4	−0.78	−0.76	−0.03	0.21	0.41	−0.26	0.21	0.80	−0.35	0.46	0.84	1.00												
Na	−0.73	−0.73	−0.05	0.08	0.35	−0.33	0.21	0.76	−0.23	0.48	0.90	0.90	1.00											
K	−0.80	−0.45	−0.07	0.21	0.20	−0.20	−0.05	0.39	−0.10	0.09	0.75	0.71	0.69	1.00										
Ca	−0.36	−0.63	0.11	−0.20	0.54	−0.56	0.35	0.86	−0.41	0.83	0.29	0.58	0.56	0.18	1.00									
Mg	−0.49	−0.72	−0.01	0.01	0.42	−0.41	0.32	0.85	−0.41	0.72	0.64	0.83	0.85	0.46	0.74	1.00								
Fe	−0.08	−0.38	0.11	−0.56	0.66	−0.62	0.59	0.21	−0.25	0.09	0.08	0.12	0.12	0.21	0.33	0.24	1.00							
Si	0.21	−0.16	−0.07	−0.41	0.29	−0.53	0.39	0.38	−0.23	0.51	−0.28	0.07	0.07	−0.15	0.67	0.34	0.40	1.00						
COLOR	−0.30	−0.61	0.09	−0.40	0.65	−0.75	0.47	0.60	−0.40	0.52	0.23	0.50	0.46	0.37	0.68	0.60	0.73	0.61	1.00					
FORST	−0.32	−0.60	−0.02	−0.39	0.70	−0.60	0.57	0.36	−0.41	0.22	0.12	0.34	0.19	0.32	0.36	0.25	0.59	0.38	0.69	1.00				
WOODL	0.02	0.01	0.14	0.00	0.05	0.06	−0.28	0.14	0.09	0.31	−0.22	−0.17	−0.12	−0.28	0.15	−0.08	−0.19	0.12	−0.02	−0.29	1.00			
TUNDR	0.63	0.76	−0.06	0.18	−0.68	0.44	−0.22	−0.69	0.34	−0.56	−0.42	−0.55	−0.53	−0.36	−0.55	−0.50	−0.26	−0.23	−0.58	−0.47	−0.53	1.00		
ORG	0.01	−0.31	−0.08	−0.26	0.29	−0.25	0.12	0.31	−0.08	0.45	−0.23	0.03	0.00	−0.27	0.34	0.07	−0.28	0.40	0.11	0.16	0.49	−0.45	1.00	
Res.LONG	0.18	−0.13	−0.14	−0.31	0.06	−0.25	0.01	0.10	−0.27	0.38	−0.29	−0.05	−0.03	−0.20	0.21	0.18	−0.22	0.27	0.03	−0.17	0.34	−0.14	0.60	1.00
NoHC	0.01	−0.33	0.01	−0.61	0.45	−0.45	0.39	−0.09	−0.14	−0.06	−0.05	−0.13	−0.03	0.05	−0.19	−0.02	0.31	−0.08	0.20	0.48	−0.16	−0.20	0.16	0.13

Summer surface-water temperature in Labrador tends to decline with increasing latitude and lake depth. The correlation (Table 4) between latitude and surface-water temperature is mostly attributable to the broad climatic gradient spanned by the study lakes. Lakes at higher latitudes will also tend to reflect a larger proportion of incoming solar radiation (Thomasson 1956), but on the other hand, days will be longer at high latitudes during summer. In addition, lake depth offers an index of lake heat-storage capacity (Shuter et al. 1983). The greater the depth of water to be heated, the lower the summer surface-water temperature. This effect should be especially apparent in those lakes that do not stratify during summer.

Given the large proportion of variance of the weighted averages explained by temperature, summer surface-water temperature must exert a strong influence upon the composition of littoral communities. The profundal community, however, is isolated for the most part from direct climatic effects. There is little doubt, therefore, that the relationship observed depends upon the great areal extent of littoral habitat in most lakes and the deposition of large numbers of littoral chironomid remains in deepwater sediments. Even in a lake having a 30-m maximum depth, 30% of the bottom habitat is likely to be shallower than 5.5 m (estimated by assuming the basin has a conical form). In warm, temperate lakes, littoral chironomid remains may dominate the fossil record, even in deep waters (Wiederholm 1979).

The distributions of chironomid taxa were demonstrated to be significantly correlated with maximum depth, as well as temperature. If the CCA had been constrained to depth alone, maximum lake depth would account for 20% of the variance explainable with all environmental variables (Table 2). Following forward selection of temperature, however, maximum lake depth could account for no more than an additional 10% of the total explainable variance. Thus, much of the influence of lake depth is probably due to its influence on surface-water temperature, discussed in the preceding paragraphs. A significant additional depth influence remains. In those lakes that stratify, the availability of cold, profundal habitat will increase relative to littoral habitat with increasing lake depth. Lake depth will also influence oxygen concentrations. Shallow lakes are more likely to develop winter anoxia. We do not have dissolved oxygen data.

The strength of relationships between chironomids, temperature, and lake depth is readily apparent in the Labrador data. For example, many chironomids common in littoral habitats of temperate latitude lakes have distributions that either do not extend beyond the tree line (Fig. 5a, 5b, 5c) or have distributions that barely extend onto the southernmost arctic tundra (Danks 1981; Walker 1991; Walker and Mathewes 1989a). These taxa are primarily temperate littoral Chironomini (i.e. Chironomini excluding *Chironomus*, *Sergentia*, and *Stictochironomus*), Zavreliina (*Stempellina*, *Stempellinella*, and *Zavrelia*), and the Pentaneurini (Fig. 5a, 5b, 5c). Although most of these "thermophilous" Chironomidae never individually compose a large proportion of the chironomid fauna, their overall relative abundance declines rapidly with increasing latitude. In Labrador this pattern is weaker for Zavreliina than for Pentaneurini or temperate Chironomini. Nevertheless, Zavreliina were rare north of 56°N. These taxa tend also to be more abundant in shallow lakes (where more littoral habitat exists and higher summer temperatures will be attained). Since temperature thresholds appear to regulate many life processes in the Chironomidae, and other aquatic insects (Pinder 1986; Walker and Mathewes 1989a; Ward and Stanford 1982), including hatching of eggs, growth and feeding by larvae, pupation, emergence, flight, and fecundity, the thermal requirements of chironomid larvae are probably key determinants of their distributions.

In contrast, relative abundance of *Heterotrissocladius* tends to decrease greatly with decreasing latitude (Fig. 5d). Because the prevalence of *Heterotrissocladius* is measured here by its relative abundance, rather than by an absolute measure (e.g. head capsules deposited per year), it is impossible to discern in our data whether the smaller proportion of *Heterotrissocladius* remains in low-latitude lakes results from a decline in its absolute abundance or alternatively from the increasing absolute abundance of other components of the benthos. Since *Heterotrissocladius* can form a dominant component of the extant chironomid fauna in profundal regions of deep temperate lakes, we believe that the decrease in its relative abundance may, in part, reflect the increased absolute abundance of "thermophilous" Chironomidae in southern Labrador. Regardless of latitude, *Heterotrissocladius* is rare or absent in all shallow Labrador lakes. Low oxygen concentrations during winter may restrict *Heterotrissocladius* and other "cold-stenothermous" chironomids to deeper lakes.

The ordination results indicate that temperature and depth are both highly correlated with axis 1. This implies that very similar faunal changes might be produced either by an abrupt change in lake depth or by a climatically induced temperature change. Despite this trend, the arctic tundra lakes are well separated in the ordination (Fig. 2b) from those of southern Labrador. The tests summarized in Table 1 also demonstrate the distinct influence of summer surface-water temperature and maximum lake depth on chironomid fauna. It should, therefore, be possible to reconstruct past water level changes, as well as water temperature fluctuations, from the fossil fauna. The possibility of confusing water level fluctuations with a climatically related temperature change must, however, be carefully evaluated in future chironomid palaeoecological studies. To minimize the possibility of misinterpretation, palaeoecologists should recognize that climatic effects should be apparent in all lakes within a region. By examining sediments from lakes that occupy shallow basins, where large water level fluctuations could not have occurred, the possibility of misinterpretation can be further reduced.

Sediment Organic Content

Most surprising was the poor, statistically insignificant correspondence between sediment organic content and composition of the chironomid fauna (Table 1). This result was obtained despite attempts to incorporate a large sediment organic content gradient. Those samples included in the CCA varied from 6.7 to 40.5% loss-on-ignition. The poor relationship between lake fauna and sediment organic content suggests that changes in lake fauna revealed by palaeolimnology should not be uncritically attributed to those changes in sediment organic content apparent in cores.

Although *Abiskomyia*, *Heterotanytarsus*, and several members of the *Harnischia* complex appear to favour mineral substrata in other studies, most chironomids, at the generic level, may not be very responsive to substrata. In addition, organic content of sediments in a core may not provide a useful indication of those substrates available for chironomids elsewhere in the lake. Chironomid remains may be transported significant

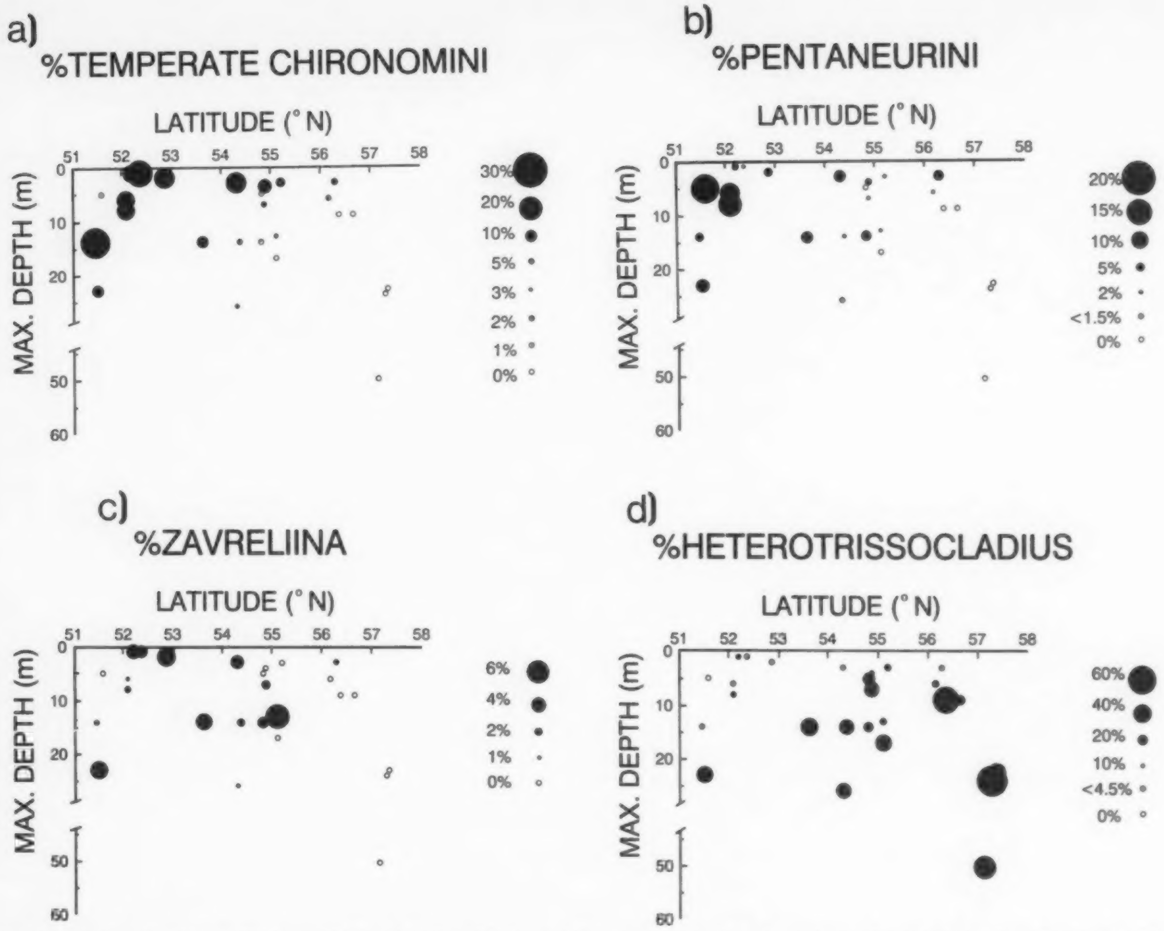

FIG. 5. Relative abundance of chironomid taxa in lakes differing with regard to latitude and maximum depth in Labrador, Canada. (a) Total temperate Chironomini; (b) total Pentaneurini, (c) total Zavreliina; (d) *Heterotrissocladius*.

distances prior to deposition. Although *Abiskomyia, Heterotanytarsus*, and *Stempellina* inhabit sand cases, these taxa are not closely grouped in our ordination.

Several studies of chironomid ecology have suggested correlations between chironomid species and substrata (e.g. McGarrigle 1980; Pinder 1986; Winnell and White 1985). Unfortunately, many of the substrate gradients studied are confounded by correlated gradients of water temperature or other environmental condition. For example, McGarrigle (1980) and Winnell and White (1985) have noted apparent substrate preferences for chironomid taxa. In both studies, however, substrate differences were correlated with depth and water temperature. Unless careful attempts are made to distinguish between the effects of related variables, such studies will not provide an accurate indication of how chironomids respond to substrate differences.

Some authors (e.g. Warner and Hann 1987) have suggested that the decline of *Heterotrissocladius* ca. 10 000 yr B.P. is related to changes in substrate, but the temporal distribution of *Heterotrissocladius* remains at Green Lake, Michigan, and Marion Lake, British Columbia, revealed through palaeolimnological analyses (Fig. 6), and our studies demonstrate that sediment organic content of cores is a poor predictor of *Heterotrissocladius* abundance. At Green Lake, the *Heterotrissocladius* decline is apparent in sediments with low organic content (Lawrenz 1975); at Marion Lake, this decline occurs within organic-rich late glacial/early Holocene sediments (Walker and Mathewes 1987a, 1989c).

The usefulness of correlations between fauna and environmental variables is best measured by their predictive ability. For example, in Ya-Ya Lake, Northwest Territories (Tsui 1980), and in Labrador, *Heterotrissocladius* is common beneath clear waters, but this genus is also common in turbid glacial lakes (Aagaard 1986; Walker and Mathewes 1989a) and was common following deforestation (ca. 1850) beneath the turbid water of the Bay of Quinte, Lake Ontario (Warwick 1980). Thus, there is little evidence that as a genus, *Heterotrissocladius* is especially responsive to suspended mineral matter.

Similarly, Walker and Mathewes (1989c) argued that Warwick's (1980, 1989) evidence, linking chironomid distributions with rates of mineral sediment accumulation, is equivocal. For Marion Lake, British Columbia, late glacial/early Holocene mineral sediment accumulation rates can be estimated from ^{14}C dates provided by Mathewes (1973, fig. 5) and bulk density, sediment dry weight, and loss-on-ignition data offered by Wainman and Mathewes (1987, fig. 1). Warwick's (1980)

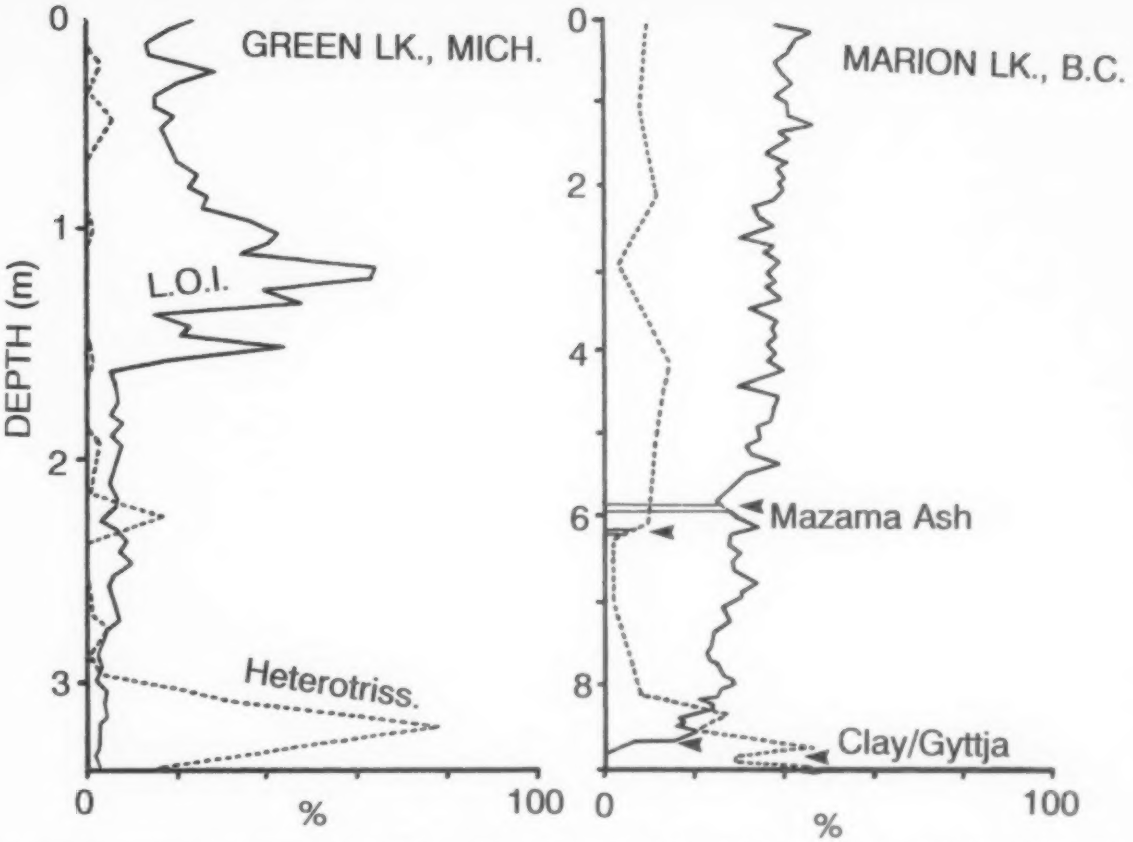

FIG. 6. Comparison of sediment loss-on-ignition and *Heterotrissocladius* profiles at Green Lake, Michigan (Lawrenz 1975), and Marion Lake, British Columbia (Walker and Mathewes 1989c). Data for Green Lake are abstracted from figures included in Lawrenz (1975). Chironomid and loss-on-ignition analyses for Marion Lake were performed on two sediment cores taken in close proximity. To permit closer correlation between these analyses, the positions of the Mazama Ash and clay/gyttja boundary are indicated for each profile. Solid lines indicate % loss-on-ignition of dry sediment; broken lines indicate relative abundance of *Heterotrissocladius*.

analysis suggests that peak *Heterotrissocladius* abundance was associated with high mineral sediment deposition (0.1–0.9 $g \cdot cm^{-2} \cdot yr^{-1}$) in the Bay of Quinte. At Marion Lake (Fig. 7), however, *Heterotrissocladius* (and other late glacial taxa) continued to be abundant for approximately 2000 yr after deposition of glacial clays, during an interval of slow mineral sediment deposition ($<0.01\ g \cdot cm^{-2} \cdot yr^{-1}$). In the Bay of Quinte, *Heterotrissocladius* never constituted more than 3% of the total fauna, but at Marion Lake, peak *Heterotrissocladius* abundance exceeds 40% of the total. The late glacial sediment accumulation rate ($\leq 0.30\ mm \cdot yr^{-1}$) at Marion Lake, and other British Columbia sites (Mathewes 1973; Walker and Mathewes 1989b), was lower than the lowest sediment accumulation rates ($0.38\ mm \cdot yr^{-1}$) inferred for any level in the Bay of Quinte core (Warwick 1980, table 8). Unfortunately, Stoermer et al. (1985, p. 762) considered the dating and sedimentation rates in the Bay of Quinte core to be based "on an undesirable degree of circular logic." The validity of Warwick's (1980) sedimentation rates might, however, be reevaluated by applying a constant-rate-of-supply ^{210}Pb dating model (e.g. Binford 1990) to Bay of Quinte radioisotopic data (provided by Kipphut 1978).

Residual Longitude

We had not anticipated that residual longitude should have had any significant influence on the chironomid fauna. Perhaps local faunistic variations occur in Labrador that are not adequately described by the other available environmental measures. Temperature and water chemistry measurements were obtained during a single visit to the lakes in 1979. They provide an imperfect assessment of local environmental differences, including temperature conditions. Local climatic conditions are especially prone to annual variations.

Fe

Differences in lake fauna also appear to be associated with variations in Fe ($P = 0.032$). In Labrador, Fe occurs in freshwaters largely in organically bound forms. Fe concentrations were positively correlated ($r = 0.73$) with water colour and were highest in the most strongly coloured lakes of southeastern Labrador. Correlations between water colour and lake fauna were noted by Thienemann (1921), and more recently by Sæther (1979). Sæther (1979) identified *Heterotanytarsus* and *Zalutschia* as indicators of humic waters. Our result (Fig. 2a) suggests a similar relationship. Evidence provided by Rasmussen and Lindegaard (1988) suggests that high Fe concentrations might influence macroinvertebrate species distributions.

Other Environmental Variables

It has been suggested that correlations also exist between lake fauna and productivity and pH (e.g. Mossberg and Nyberg

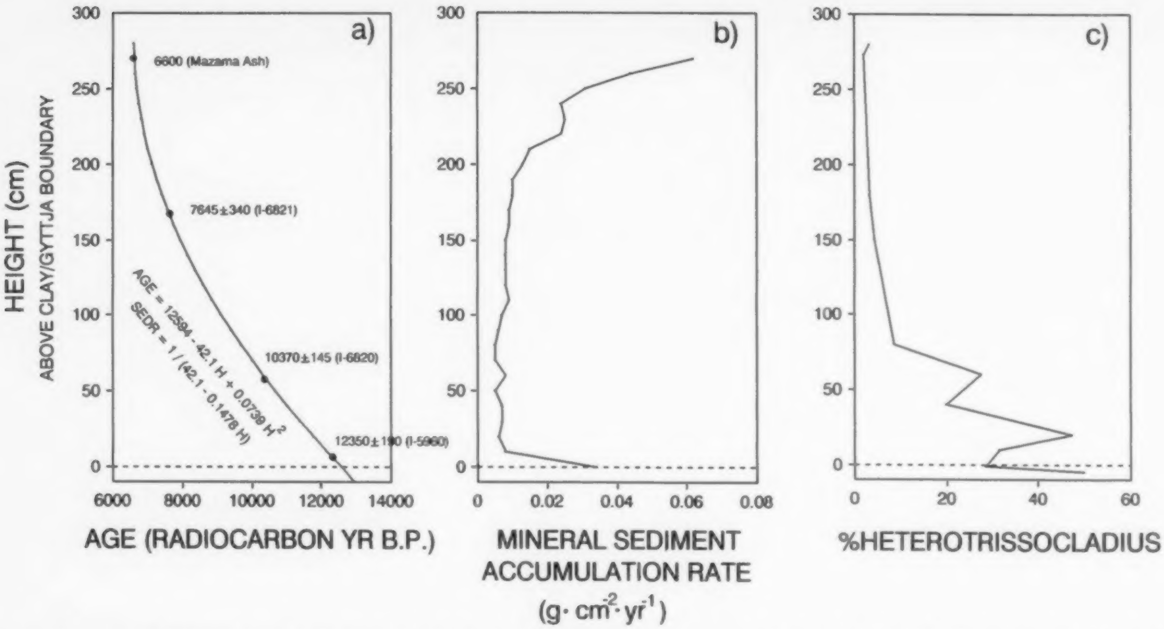

FIG. 7. Comparison of late glacial/early Holocene (a) depth–time, (b) mineral sediment accumulation rate, and (c) % *Heterotrissocladius* profiles for Marion Lake, British Columbia. Depths in sediment are measured as heights relative to the clay/gyttja boundary, facilitating comparisons among profiles (obtained using three cores taken in close proximity). Depth–time relationships are modelled by the regression equation AGE = 12594 − 42.1 H + 0.0739 H^2 where H is the height (cm) above the clay/gyttja boundary. Whole sediment accumulation rates (SEDR (cm·yr^{-1}) are derived from the derivative of the regression line SEDR = $-[d(\mathrm{AGE})/d(\mathrm{H})]^{-1}$. Mineral sediment accumulation rates (MSEDR (g·cm^{-2}·yr^{-1})) were derived from the relationship MSEDR = SEDR × DRYWT × BD × (100% − LOI)/100% where DRYWT (sediment dry weight (g dry·g wet^{-1})), BD (bulk density (g wet·cm^{-3})), and LOI (% loss-on-ignition) were estimated at 10-cm intervals from Wainman and Mathewes' (1987) illustration.

TABLE 5. Summary statistics for environmental variables measured at the 24 lakes included in the exploratory CCA.

Variable	(Weighted) mean	SD	Minimum	Maximum
Elevation (m)	405.70	158.47	43.00	587.00
Latitude (°N)	54.59	1.81	51.45	57.33
Res. longitude (°W)	0.14	0.90	−2.16	1.56
Lake area (ha)	95.63	41.07	54.00	189.00
Depth (m)	12.29	10.62	1.00	50.00
Organic content (% of dry wt.)	23.69	9.31	6.70	40.50
Temperature (°C)	12.23	2.31	7.60	18.00
Secchi depth (m)	6.75	4.40	1.00	15.00
Colour (Pt units)	21.80	32.25	5.00	130.00
pH	6.38	0.27	5.81	6.92
Conductance (μs·cm^{-1})	9.72	6.91	1.00	27.00
Alkalinity (μeq·L^{-1})	52.75	30.54	9.70	115.90
Cl (mg·L^{-1})	0.60	1.11	0.04	4.90
SO_4 (mg·L^{-1})	0.96	0.43	0.44	2.43
Na (mg·L^{-1})	0.74	0.72	0.24	3.70
K (mg·L^{-1})	0.16	0.10	0.03	0.49
Ca (mg·L^{-1})	1.23	0.51	0.48	2.21
Mg (mg·L^{-1})	0.26	0.17	0.07	0.69
Fe (mg·L^{-1})	0.23	0.62	0.05	3.03
Total Si (mg·L^{-1})	0.94	0.78	0.05	2.89
Total P (μg·L^{-1})	14.97	19.16	2.00	74.00
Forest[a]	0.20	0.40		
Woodland[a]	0.25	0.43		
Tundra[a]	0.46	0.50		

[a] Variables entered as binary codes.

1979; Sæther 1979). In this study, no effect was demonstrated, but no attempt was made to incorporate large pH or trophic gradients in our analysis (Table 5). Only three lakes had total P concentrations $>25\ \mu g \cdot L^{-1}$. Total P concentrations tend to be correlated with summer surface-water temperature (r = 0.71). Lakewater pH ranged from 5.8 to 6.9. If attempts were made to sample broader pH and trophic gradients, we expect that effects from these influences would be more apparent. Since much of the P in Labrador lakes is organically bound, not available to primary producers, a more appropriate measure of trophic state would be soluble reactive P.

Secchi depth was not selected as a significant variable by forward selection in CCA. However, prior to forward selection, this variable could have accounted for 20% of the variance explained by all environmental variables (Table 2). We had no reason to believe that chironomid assemblages were responding to water transparency. It should be noted that at 12 of the 24 lakes analysed in our exploratory CCA, the Secchi disk was visible while resting on the lake bottom. Thus, the measured Secchi depths were not a good measure of water transparency and tended to be correlated with lake depth (r = 0.79). Since water colour is greatest in humic lakes of southern Labrador, Secchi depth also tends to be correlated with latitude (r = 0.59) and surface-water temperature (r = −0.69). Thus, we have no reason to believe that the correlation between measured Secchi depth and fauna has biological significance. Following forward selection of temperature, Secchi depth could have explained only an additional 6% of the total variance explainable by all environmental variables.

The exploratory CCA that we report here describes the result obtained if one variable, "number of head capsules" (NoHC), is excluded from the analysis. A preliminary partial CCA had revealed no significant effect (P = 0.22) from this variable, once the effects of temperature and depth (entered as covariables) had been removed. If NoHC is included as a variable in the exploratory CCA, however, temperature, residual longitude, and NoHC are selected. NoHC is negatively correlated with maximum lake depth and positively related to presence of forest and temperature (Table 4). It seems likely that each of these variables influences the production of Chironomidae and thus the concentration of chironomid remains in each sample. This possibility requires further study.

Reliability of Chironomid-Inferred Temperatures

The weighted-averaging model developed for the Labrador chironomid–temperature relationship provided predicted summer surface-water temperatures that are strongly correlated with actual Labrador temperatures (Fig. 3). Nevertheless, the strength of this correlation is inflated somewhat by the circularity inherent to predicting temperatures for the same lakes used in developing the model.

A more rigorous test was provided by attempts to infer summer surface-water temperatures for British Columbia lakes from the Labrador model. We had anticipated that a significant correlation between predicted temperatures and altitude of British Columbia lakes should exist. The positive result (Fig. 4) confirms our observation that the chironomid fauna changes in a similar manner with increasing elevation in British Columbia as with increasing latitude in Labrador.

In fact, the correlation is especially striking, since the weighted-averaging model was developed on the opposite coast of the North American continent! Although tree pollen is widely recognized as a good palaeoclimatic indicator, no palynologist would expect to be able to infer palaeoclimates in British Columbia if his/her entire knowledge of tree ecology was limited to observations of tree species in Labrador. Consequently, we believe that the Chironomidae may prove to be the more robust palaeoclimatic indicators for arctic/alpine and subarctic/subalpine environments.

TABLE 6. Mean squared residual distance from fit with the first two axes generated by the temperature-constrained CCA.

Region in which samples were collected	Mean squared residual distance		Number of samples
	Axis 1	Axis 2	
Labrador	0.73	0.52	26
British Columbia	8.36	7.88	19
Devon Island, N.W.T.	2.42	2.27	4
Western subarctic and Low arctic, N.W.T.	1.41	1.17	15
Splan Lake, N.B. (late glacial)	1.19	1.02	12

Aquatic organisms have a shorter life span than trees, and most have efficient means for dispersal, and they are independent of pedogenic processes. Thus, aquatic organisms are able to respond more rapidly to climatic change than terrestrial vegetation (e.g. Ammann 1989; Smol et al. 1990). Furthermore, tree pollen is often transported long distances by wind, contaminating sediments beyond their range. This is especially a problem in arctic and alpine lakes where local vegetation is scant and produces little pollen (Lamb 1984). In contrast, unless a large river enters the lake, the head capsules of chironomid larvae are entirely locally produced.

Although the changes in fauna with increasing elevation in British Columbia are similar to the latitude-related differences in Labrador, several distinct differences in fauna are apparent between these regions, including the absence of *Abiskomyia* and *Oliveridia* in British Columbia, the greater relative abundance of *Tanytarsus* s. lat. in high-elevation British Columbia lakes, and the presence of several taxa in British Columbia that were not found in Labrador. Thus the Labrador lakes provide imperfect analogues for British Columbia fauna.

The reliability of inferences from weighted-averaging calibration is influenced by the fit of samples to the constrained CCA axis (Birks et al. 1990). Squared residual distances from fit were included in the CANOCO output. These distances were high for many British Columbia lakes, relative to distances obtained for Labrador lakes and passive samples from other Canadian regions (Table 6). It appears that the difference in fauna between British Columbia and Labrador is reflected by these residual distances. The Labrador temperature-constrained CCA provides a better model for fauna in lakes of the western Canadian arctic and subarctic and for the late glacial fauna of a New Brunswick lake (Table 6).

More accurate quantitative palaeotemperature reconstructions will be possible once the range of thermal conditions represented in the Labrador regression data is expanded to include data from the Canadian arctic archipelago and Canadian regions south of Labrador. This work is presently in progress (e.g. Walker 1991). In the present weighted-averaging-regression data, summer surface-water temperatures range from 7.6 to 18°C. All temperatures inferred from the Labrador weighted-averaging model will be constrained within this range. In British Columbia the summer surface-water temperature of small, low-elevation lakes may exceed 20°C whereas temperatures in glacial lakes may never exceed 5°C (Hamilton 1965; Walker and Mathewes 1989a, 1989b). Consequently, temperatures for many low-elevation lakes are certain to be underestimated whereas the temperatures predicted for some glacial lakes are probably overestimated.

If postglacial palaeotemperatures were inferred for lakes using the present model, the same limitation would be apparent. By expanding our present surface sample data set, we can eliminate this problem and will also be able to provide a more complete evaluation of errors associated with chironomid-inferred palaeotemperature estimates.

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References

Aagaard, K. 1986. The chironomid fauna of north Norwegian lakes, with a discussion on methods of community classification. Holarct. Ecol. 9: 1–12.

Ammann, B. 1989. Response times in bio- and isotope-stratigraphies to late-glacial climatic shifts — an example from lake deposits. Eclogae Geol. Helv. 82: 183–190.

American Public Health Association. 1980. Standard methods for the examination of water and wastewater. 15th ed. Washington, DC. 1134 p.

Binford, M. W. 1990. Calculation and uncertainty analysis of ^{210}Pb dates for PIRLA project lake sediment cores. J. Paleolimnol. 3: 253–267.

Birks, H. J. B., J. M. Line, S. Juggins, A. C. Stevenson, and C. J. F. ter Braak. 1990. Diatoms and pH reconstruction. Philos. Trans. R. Soc. Lond. B Biol. Sci. 327: 263–278.

Boubée, J. A. T. 1983. Past and present benthic fauna of Lake Maratoto with special reference to the Chironomidae. Ph.D. thesis, University of Waikato, Hamilton, N.Z. 251 p.

Brundin, L. 1951. The relation of O_2-microstratification at the mud surface to the ecology of the profundal bottom fauna. Inst. Freshwater Res. Drottningholm Rep. 32: 32–42.

Chessel, D., J. D. Lebreton, and N. Yoccoz. 1987. Propriétés de l'analyse canonique des correspondances; une illustration en hydrobiologie. Rev. Stat. Appl. 35: 55–72.

Clair, T. A., D. R. Engstrom, and W. Whitman. 1982. Water quality of the surface waters of Newfoundland and Labrador. Environ. Can. Data Rep. IWD-AR-WQB-82-83, Inland Waters Directorate, Moncton, N.B. 55 p.

Cook, E. F. 1956. The Nearctic Chaoborinae (Diptera: Culicidae). Bull. Univ. Minn. Agr. Exp. Stn. 218: 102 p.

Danks, H. V. 1981. Arctic arthropods: a review of systematics and ecology with particular reference to the North American fauna. Entomological Society of Canada, Ottawa, Ont. 608 p.

de Bakker, A. J. 1989. Effects of ammonia emission on epiphytic lichen vegetation. Acta Bot. Neerl. 38: 337–342.

DeCosta, J. J. 1964. Latitudinal distribution of chydorid Cladocera in the Mississippi Valley, based on their remains in surficial lake sediments. Invest. Ind. Lakes Streams 6: 65–101.

Dixit, S. S., A. S. Dixit, and J. P. Smol. 1989. Relationship between chrysophyte assemblages and environmental variables in seventy-two Sudbury lakes as examined by canonical correspondence analysis (CCA). Can. J. Fish. Aquat. Sci. 46: 1667–1676.

Douglas, M. S. V., and J. P. Smol. 1987. Siliceous protozoan plates in lake sediments. Hydrobiologia 154: 13–23.

Engstrom, D. R. 1987. Influence of vegetation and hydrology on the humus budgets of Labrador lakes. Can. J. Fish. Aquat. Sci. 44: 1306–1314.

Fängström, I., and E. Willén. 1987. Clustering and canonical correspondence analysis of phytoplankton and environmental variables in Swedish lakes. Vegetatio 71: 87–95.

Frey, D. G. 1988. Littoral and offshore communities of diatoms, cladocerans and dipterous larvae, and their interpretation in paleolimnology. J. Paleolimnol. 1: 179–191.

Gannon, J. E. 1971. Two counting cells for the enumeration of zooplankton micro-crustacea. Trans. Am. Microsc. Soc. 90: 486–490.

Hamilton, A. L. 1965. An analysis of a freshwater benthic community with special reference to the Chironomidae. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

Hann, B. J., and B. G. Warner. 1987. Late Quaternary Cladocera from coastal British Columbia, Canada: a record of climatic or limnologic change? Arch. Hydrobiol. 110: 161–177.

Johnson, R. K., and T. Wiederholm. 1989. Classification and ordination of profundal macroinvertebrate communities in nutrient poor, oligo-mesohumic lakes in relation to environmental data. Freshwater Biol. 21: 375–386.

Kingston, J. C., and H. J. B. Birks. 1990. Dissolved organic carbon reconstructions from diatom assemblages in PIRLA project lakes, North America. Philos. Trans. R. Soc. Lond. B Biol. Sci. 327: 279–288.

Kipphut, G. W. 1978. An investigation of sedimentary processes in lakes. Ph.D. thesis, Columbia University, New York, NY. 180 p.

Lamb, H. F. 1984. Modern pollen spectra from Labrador and their use in reconstructing Holocene vegetational history. J. Ecol. 72: 37–59.

Lawrenz, R. W. 1975. The developmental paleoecology of Green Lake, Antrim County, Michigan. M.Sc. thesis, Central Michigan University, Mount Pleasant, MI.

Mathewes, R. W. 1973. A palynological study of postglacial vegetation changes in the University Research Forest, southwestern British Columbia. Can. J. Bot. 51: 2085–2103.

McGarrigle, M. L. 1980. The distribution of chironomid communities and controlling sediment parameters in L. Derravaragh, Ireland, p. 275–282. *In* D. A. Murray [ed.] Chironomidae: ecology, systematics, cytology, and physiology. Pergamon Press, Oxford.

Meriläinen, J. 1967. The diatom flora and the hydrogen-ion concentration of the water. Ann. Bot. Fenn. 4: 51–58.

Moser, K. A. 1988. A palaeoecological investigation of the treeline zone north of Yellowknife, NWT. M.Sc. thesis, McMaster University, Hamilton, Ont. 153 p.

Mossberg, P., and P. Nyberg. 1979. Bottom fauna of small acid forest lakes. Inst. Freshwater Res. Drottningholm Rep. 58: 77–87.

Oliver, D. R., and M. E. Roussel. 1983. The insects and arachnids of Canada. Part 11. The genera of larval midges of Canada; Diptera: Chironomidae. Agric. Can. Publ. 1746: 263 p.

Pinder, L. C. V. 1986. Biology of freshwater Chironomidae. Annu. Rev. Entomol. 31: 1–23.

Rasmussen, K., and C. Lindegaard. 1988. Effects of iron compounds on macroinvertebrate communities in a Danish lowland river system. Water Res. 22: 1101–1108.

Rowe, J.S. 1972. Forest regions of Canada. Can. For. Serv. Publ. 1300: 172 p.

Sæther, O. A. 1970. Nearctic and Palaearctic *Chaoborus* (Diptera: Chaoboridae). Bull. Fish. Res. Board Can. 174: 58 p.

1979. Chironomid communities as water quality indicators. Holarct. Ecol. 2: 65–74.

Shuter, B. J., D. A. Schlesinger, and A. P. Zimmerman. 1983. Empirical predictors of annual surface water temperature cycles in North American lakes. Can. J. Fish. Aquat. Sci. 40: 1838–1845.

Smol, J. P., I. R. Walker, and P. R. Leavitt. 1990. Paleolimnology and hindcasting climatic trends. Verh. Int. Ver. Limnol. 24. (In press)

Stevenson, A. C., H. J. B. Birks, R. J. Flower, and R. W. Battarbee. 1989. Diatom-based pH reconstruction of lake acidification using canonical correspondence analysis. Ambio 18: 228–233.

Stoermer, E. F., J. A. Wolin, C. L. Schelske, and D. J. Conley. 1985. Postsettlement diatom succession in the Bay of Quinte, Lake Ontario. Can. J. Fish. Aquat. Sci. 42: 754–767.

Synerholm, C. C. 1979. The chydorid cladocera from surface lake sediments in Minnesota and North Dakota. Arch. Hydrobiol. 86: 137–151.

ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67: 1167–1179.

1987a. CANOCO — a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1).

TNO Institute of Applied Computer Science, Statistical Department, Wageningen, The Netherlands. 95 p.

1987b. Calibration, p. 78–90. *In* R. H. G. Jongman, C. J. F. ter Braak, and O. F. R. van Tongeren [ed.] Data analysis in community and landscape ecology. Pudoc, Wageningen. 299 p.

1987c. Partial canonical correspondence analysis, p. 83–91. *In* C. J. F. ter Braak. Unimodal models to relate species to environment. Ph.D. thesis, University of Wageningen, Wageningen, The Netherlands.

1989. CANOCO version 3.0. unpublished program. (Version 3.1 available from Microcomputer Power, 111 Clover Lane, Dep. UC3, Ithaca, NY 14850-4930, USA)

ter Braak, C. J. F., and C. W. N. Looman. 1987. Regression, p. 29–77. *In* R. H. G. Jongman, C. J. F. ter Braak, and O. F. R. van Tongeren [ed.] Data analysis in community and landscape ecology. Pudoc, Wageningen. 299 p.

ter Braak, C. J. F., and H. van Dam. 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia 178: 209–223.

Thienemann, A. 1921. Seetypen. Naturwissenschaften 9: 343–346.

Thomasson, K. 1956. Reflections on arctic and alpine lakes. Oikos 7: 117–143.

Tsui, P. T. P. 1980. Zoobenthos, p. 163–197. *In* P. J. McCart [ed.] Effects of siltation on the ecology of Ya-Ya Lake, N.W.T. Environmental Studies No. 13. Northern Affairs Program, Publ. No. QS-8240-000-EE-A1, Ottawa, Ont.

Uutala, A. J. 1990. *Chaoborus* (Diptera: Chaoboridae) mandibles — paleolimnological indicators of the historical status of fish populations in acid-sensitive lakes. J. Paleolimnol. 4: 139–151.

Wainman, N., and R. W. Mathewes. 1987. Forest history of the last 12 000 years based on plant macrofossil analysis of sediment from Marion Lake, southwestern British Columbia. Can. J. Bot. 65: 2179–2187.

Walker, I. R. 1988. Late-Quaternary palaeoecology of Chironomidae (Diptera: Insecta) from lake sediments in British Columbia. Ph.D. thesis, Simon Fraser University, Burnaby, B.C.

1991. Modern assemblages of arctic and alpine Chironomidae as analogues for late-glacial communities. Hydrobiologia. (In press)

Walker, I. R., and R. W. Mathewes. 1987a. Chironomidae (Diptera) and postglacial climate at Marion Lake, British Columbia, Canada. Quat. Res. 27: 89–102.

1987b. Chironomids, lake trophic status, and climate. Quat. Res. 28: 431–437.

1989a. Chironomidae (Diptera) remains in surficial lake sediments from the Canadian Cordillera: analysis of the fauna across an altitudinal gradient. J. Paleolimnol. 2: 61–80.

1989b. Early postglacial chironomid succession in southwestern British Columbia, Canada, and its paleoenvironmental significance. J. Paleolimnol. 2: 1–14.

1989c. Much ado about dead Diptera. J. Paleolimnol. 2: 19–22.

Ward, J. V., and J. A. Stanford. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annu. Rev. Entomol. 27: 97–117.

Warner, B. G., and B. J. Hann. 1987. Aquatic invertebrates as paleoclimatic indicators? Quat. Res. 28: 427–430.

Warwick, W. F. 1980. Palaeolimnology of the Bay of Quinte, Lake Ontario: 2800 years of cultural influence. Can. Bull. Fish. Aquat. Sci. 206: 118 p.

1989. Chironomids, lake development and climate: a commentary. J. Paleolimnol. 2: 15–17.

Webb, R. S., and T. Webb, III. 1988. Rates of sediment accumulation in pollen cores from small lakes and mires of eastern North America. Quat. Res. 30: 284–297.

Whiteside, M. C. 1970. Danish chydorid Cladocera: modern ecology and core studies. Ecol. Monogr. 40: 79–118.

Wiederholm, T. 1979. Chironomid remains in recent sediments of Lake Washington. Northwest Sci. 53: 251–256.

Wiederholm, T. [ed.] 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part 1 — Larvae. Entomol. Scand. Suppl. 19: 457 p.

Winnell, M. H., and D. S. White. 1985. Ecology of some Chironomidae (Diptera) from southeastern Lake Michigan, U.S.A. Trans. Am. Entomol. Soc. 111: 279–359.

Wright, H. E. Jr. 1990. An improved Hongve sampler for surface sediments. J. Paleolimnol. 4: 91–92.

Effect of Smolt Size on Smolt-to-Adult Survival for Chilko Lake Sockeye Salmon (*Oncorhynchus nerka*)

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Three approaches were used to test the hypothesis that smolt-to-adult survival is independent of smolt size for Chilko Lake sockeye salmon (*Oncorhynchus nerka*). The mean distance between the focus of the scale and the first annulus, a reliable indicator of smolt size, was greater for adult scales than for smolt scales from the same brood year in two of the three years we examined. This indicated a higher smolt-to-adult survival for larger smolts in these brood years. The abundance of smolts of different fork lengths, based on back-calculation procedures from adult scales, was compared with the abundance of smolts of different fork lengths at the time of outmigration within brood years. In all three years studied, there was a two- to threefold increase in smolt-to-adult survival as smolt length increased. However, there was no significant relationship between smolt-to-adult survival and mean annual smolt fork length based on a 34-yr time series; this lack of relationship was probably caused by limited variation in mean annual smolt fork length over the 34-yr period and other variables, independent of smolt size, that affect survival and exhibit considerable interannual variation.

On s'est servi de trois approches différentes pour vérifier chez le saumon rouge (*Oncorhynchus nerka*) du lac Chilko l'hypothèse selon laquelle la survie du smolt jusqu'à l'âge adulte ne dépend pas de la grosseur du poisson au stade de smolt. La distance moyenne entre le foyer et le premier anneau de l'écaille, indicateur fiable de la grosseur du smolt, était plus élevée sur les écailles des adultes que sur celles du smolt de la même génération pour deux des trois années que nous avons étudiées. Ce fait signifie que ce sont les poissons les plus gros au stade de smolt qui ont survécu en plus grand nombre jusqu'à l'âge adulte dans ces générations. On a comparé l'abondance du smolt de différentes longueurs à la fourche, déterminées d'après les écailles à l'âge adulte, avec l'abondance du smolt de différentes longueurs à la fourche à l'époque de la migration vers l'océan dans une même génération. On a observé pour les trois années une augmentation du double ou du triple de la survie du smolt jusqu'à l'âge adulte en fonction de l'accroissement de la longueur du smolt. Toutefois, l'analyse d'une série chronologique de 34 ans n'a révélé aucune relation significative entre la survie du smolt jusqu'à l'âge adulte et la moyenne annuelle de la longueur à la fourche; l'absence de relation était probablement une conséquence de la variation limitée de la moyenne annuelle de la longueur à la fourche du smolt pendant la période de 34 ans étudiée et de l'influence sur la survie d'autres variables, indépendantes de la grosseur du smolt, et variant interannuellement dans une mesure considérable.

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A characteristic feature of most salmon populations is the large variation in survival among brood years for the period from the time the fish migrate to sea as juveniles to the time they return to inshore areas as adults. In part, the variation in survival may be explained by differences in the size of juveniles at the time of their seaward migration. Evidence for size-dependent survival at some localities during the latter period of freshwater residence and the early period of ocean residence has been provided for chum (*Oncorhynchus keta*) (Parker 1971; Healey 1982; Hargreaves and LeBrasseur 1986) and pink salmon (*O. gorbuscha*) (Parker 1971). Evidence for size-dependent survival between the time they move into the ocean environment as juveniles and the time they return to freshwater as adults is suggested for coho (*O. kisutch*) (Johnson 1970; Hager and Noble 1976; Bilton et al. 1982), chum and pink salmon (Kobayashi 1980), and steelhead trout (*O. mykiss*) (Ward et al. 1989).

The degree to which smolt-to-adult survival in sockeye salmon (*O. nerka*) (i.e. the period between the time they leave the nursery lake as juveniles and the time they return to inshore areas as adults) depends on juvenile size is, however, less certain. Foerster (1954) showed for the small sockeye salmon stock from Cultus Lake in the lower Fraser River watershed that smolt-to-adult survival was positively correlated with mean smolt size. Subsequently, Macdonald et al. (1987) suggested that for Babine Lake sockeye, smolt-to-adult survival within brood years increased with increases in smolt size-class based on the application of coded wire tags to smolts and the recovery of tags in adults. However, the results of this study were equivocal, as they also found greater mortality in the smallest smolt size-classes as the direct result of tagging. Ricker (1962) speculated that smolt-to-adult survival increased with increases in mean smolt size based on pooling data from six sockeye stocks from British Columbia and Alaska that had a combined range in smolt fork length from 70 to 170 mm. However, this relationship was not always evident within individual stocks. Hyatt and Stockner (1985) did not find any effect of mean smolt size on smolt-to-adult survival when combining data from four sockeye salmon stocks originating in coastal areas of southern British Columbia. Finally, Koenings and Burkett (1987), using data from nine Alaskan lakes, proposed a curvilinear relationship between smolt size and smolt-to-adult survival in which survival increased with fork length up to a smolt size of approximately 120 mm and then decreased for larger smolt sizes.

The five studies cited above do not suggest any consistent relationship between smolt size and smolt-to-adult survival for sockeye salmon. Further, all but one study was based on the measure mean smolt size. As a result, it was not possible to determine how changes in smolt-to-adult survival varied among different size-classes of smolts originating from the same lake system and year.

The objective of this study was to test the hypothesis that smolt-to-adult survival of Chilko Lake sockeye salmon was independent of smolt size. We used three independent techniques to test the hypothesis. The first was a comparison between smolt-to-adult survival rate and mean smolt fork length for a 34 brood year time series. The second and third approaches involved within brood year comparisons. The second approach was a comparison of the mean value of a scale characteristic that was an index of smolt size between smolts and adults from the same brood year. The third approach, based on the same scale characteristic, was a comparison of smolt-to-adult survival rates for different smolt size-classes from the same brood year.

Methods

The Chilko Lake sockeye salmon stock, originating from the upper portion of the Fraser River watershed, is the second largest sockeye stock in the system (Cass 1989). Total annual adult return and smolt production of Chilko sockeye for the period from brood year 1952 to 1985 exhibits a cyclic pattern with peaks in abundance generally occurring once every 4 yr (Fig. 1). The total adult return in the years of greatest abundance is generally between 2 and 3 million fish. The total return in the smallest of the intervening years is usually less than 500 000 fish. Most Chilko sockeye (95%) smolt at 1 yr of age and the remainder (5%) at 2 yr of age (1949–86 average). Ninety-two percent of the adults return at 4 yr of age. The remaining adults return as 3-yr-old (1%) and 5-yr-old fish (7%) (1948–84 average). The analyses reported herein are restricted to 1- and 4-yr-old smolts and adults, respectively.

Annual estimates of the size and abundance of smolts and adults and scale samples from both smolts and adults from Chilko Lake are available for the brood years 1952–85 from the files of the Pacific Salmon Commission. We used data from all brood years to determine the relationship between smolt-to-adult survival and mean smolt fork length. Analyses of size-dependent smolt-to-adult survival within broods were based on data from the 1971, 1976, and 1977 brood years. These three brood years were chosen because of the availability of scale measurements and independent of any consideration of smolt size or abundance.

Daily estimates of smolt abundance were made at the outlet of Chilko Lake throughout the period of outmigration (April–May) each year from 1952 to 1985 (Goodlad et al. 1974).

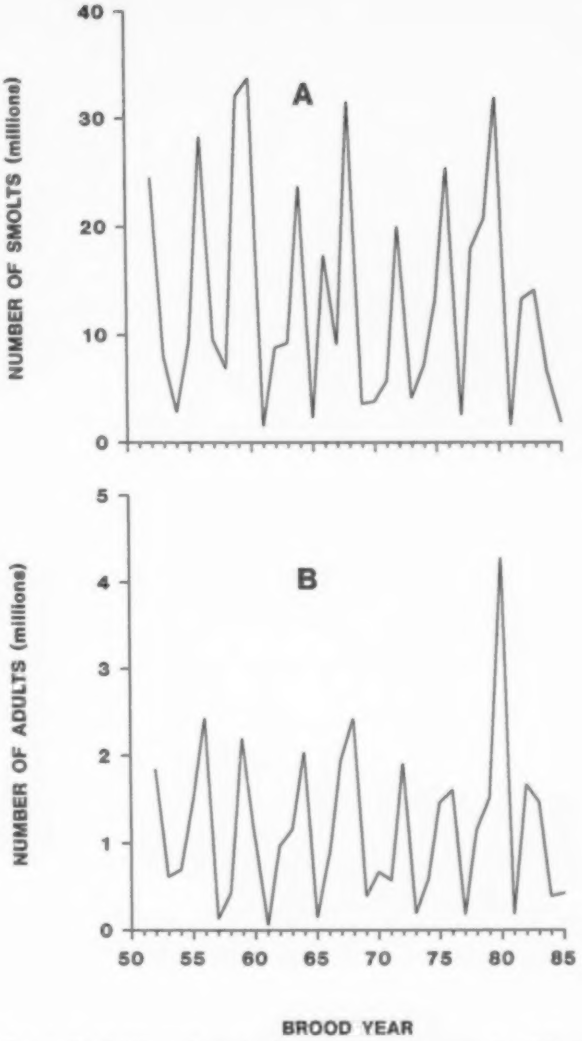

FIG. 1. Total (A) smolt production and (B) adult returns for Chilko Lake sockeye salmon for brood years 1952–85.

The fork lengths of approximately 300 smolts/day were measured to the nearest millimetre during the outmigration. Each daily estimate of mean fork length was weighted by the proportion of the total smolt outmigration leaving the lake that day. The weighted estimates were then summed to determine the seasonal mean smolt size. Scales and fork length measurements were collected from an additional random sample of approximately 100 fish two or three times each year at the peak periods of outmigration according to the methods of Clutter and Whitesel (1956).

Scale samples were obtained from adults on the primary spawning grounds located in the Chilko River at the north end of the lake. Adult scale samples were also collected in 1971 and 1976 from a small stock at the south end of the lake. All scales were collected according to the methods of Clutter and Whitesel (1956). The sampling procedure involved walking transects across the river or removing fish from eddies. In both cases, the first 120 dead males and 120 dead females seen were sampled. The procedure, designed to produce a representative sample of the entire population, was repeated three times each year during the period of spawning. The samples were collected at approximately the time of 10, 50, and 90% die-off.

Clutter and Whitesel (1956) showed that various measures of scale size and number of circuli on scales are highly correlated with body size in sockeye salmon smolts. We needed to identify one such scale characteristic that could be used to back-calculate the size of smolts from adult scales. Four measures of smolt scale size, the radius from the focus to the outer perimeter of the scale (R1), the radius from the focus to the first circulus of the annular ring (R2), and the total number of circuli in these two growth zones (C1 and C2, respectively) were made from a magnified image (255 ×) of an acetate impression of each scale for the 1971 brood year. We chose to use scale characteristic R2 for the back-calculation procedure because of the highly significant correlation coefficient between R2 and smolt size ($r = 0.766$, *t*-test, $P < 0.001$) and because it could be unequivocally identified in both smolt and adult scales.

The age structure of the smolt population was determined from scales whereas the age structure of the adult population sampled on the spawning grounds was determined from otoliths.

Inferences can be made about size-dependent smolt-to-adult survival by comparing the frequency distributions of scale characteristic R2 at the smolt and adult stages providing (i) smolts sampled for scales represent the relative proportion of the different size-classes of smolts in the entire population and (ii) adult scale samples are representative of the entire adult population in terms of scale characteristic R2.

We tested the representativeness of the smolt samples by comparing the composite length–frequency distribution of the age 1 smolts sampled during peak migration dates (dates of scale samples) with the composite length–frequency distribution based on samples collected throughout the smolt outmigration for each of the three brood years (Fig. 2). The composite length–frequency distribution for the latter was generated by weighting the daily length–frequency distribution by the proportion of the total smolt run that moved from the lake each day. The daily length–frequency distributions were then summed by size-class to form composite distributions. The composite distributions based on samples collected only during the peak periods of outmigration were generated in the same manner except that the summing operation only involved the two or three sampling dates. The frequency distribution of smolt fork length over the entire period of the outmigration based on length measurements made only on those dates when scale samples were taken was not significantly different (chi-square, $P > 0.05$) from the distribution based on daily length measurements for each of the three brood years. Further, the absolute difference between the means of the two distributions for each of the three brood years, 0.29, 0.51, and 0.80 mm for 1971, 1976, and 1977, respectively, was small. Consequently, we concluded that the smolts sampled for scales adequately represented the total smolt population with respect to fork length.

One reason that adult scale samples taken on the spawning grounds may not be representative of the entire adult population is the size-selective nature of gillnet fisheries (Todd and Larkin 1971; Ricker 1982). An average of 69% of the total catch of Chilko sockeye salmon from 1971, 1976, and 1977 brood years was taken by gill nets, with the remainder harvested by purse seines (Starr et al. 1984). The size-selective nature of the gillnet fishery would bias estimates of size-dependent survival if scale characteristic R2, which is a good index of smolt size, was also correlated with adult body size. This is a necessary result, as the distribution associated with scale characteristic R2 from adult fish found on the spawning grounds, the fish from which scale samples were taken, would be different from the distribution of the unfished population. Correlation between adult body size and scale characteristic R2 for Chilko sockeye salmon sampled on the spawning grounds resulted in coefficients of 0.071, 0.117, and 0.009 for the 1971, 1976, and 1977 brood years, respectively. Consequently, we concluded that adult size varied independently of scale characteristic R2 and assumed that any size-selective effects of the commercial gillnet fishery would not alter the distribution of scale characteristic R2 between the fished and unfished population.

The representativeness of the scale samples could also be affected by the age structure of the adult sockeye salmon population. Adult sockeye return to the Chilko system as both 4- and 5-yr-old fish. However, the adult scales we used to estimate the size of adults as smolts came only from 4-yr-old fish. Although present, the contribution of 5-yr-old Chilko sockeye to the total return was small, ranging from 0.0 to 1.4% for the 1971, 1976, and 1977 brood years. Consequently, we assume that omitting scales from 5-yr-old fish will have a negligible effect on the interpretation of our results.

Sockeye salmon spawn in both the south end of Chilko Lake and in the Chilko River at the north end of the lake. Since scales from only the northern population were used to make inferences about size-dependent survival, it was important to identify the contribution of the southern spawners to the smolt population in Chilko Lake. Estimates of the size of the spawning population of the two stocks based on mark–recapture experiments indicated that the northern population accounted for 94–96% of the total spawning stock (i.e. north- and south-end populations combined) for the 1971, 1976, and 1977 brood years (Cass 1989). We used a maximum likelihood mixture model (Fournier et al. 1984) to estimate the contribution of the two populations as a check on the estimates of spawning escapement derived from the mark–recapture studies. The maximum likelihood mixture model used data from adult scales from both the northern and southern populations for the 1971 and 1976 brood years (scales were not available for the south-end population for the 1977 brood year). We compared the frequency distribution of circuli in the freshwater growth zone (C1) from both populations of adults in 1975 and 1980 with the frequency distribution of circuli of smolts from the 1973 and 1978 outmigrations, respectively, to determine the contribution of the two populations to the Chilko Lake smolt outmigrations. C1 was used in place of R2, as measurements of R2 were not available for the lake-spawning population.

The number of smolts in each 1-mm fork length size-class for the 1971, 1976, and 1977 brood years was determined by multiplying the relative proportion of smolts in each size-class by the estimate of the total number of smolt outmigrants. The number of adults that returned from each smolt size-class was determined by multiplying the proportion of adults that had scale characteristic R2 corresponding to a particular smolt size-class by the total adult return. Estimates of total adult return (catch plus spawning escapement) were from Cass (1989). Although it is not possible to determine the confidence limits on the estimates of total run size, the estimates are believed to be accurate (J. C. Woodey, Pacific Salmon Commission, Vancouver, B.C., pers. comm.). Chilko Lake sockeye salmon comigrate with a small number of other sockeye salmon stocks through the commercial fisheries. Each of these stocks tends to

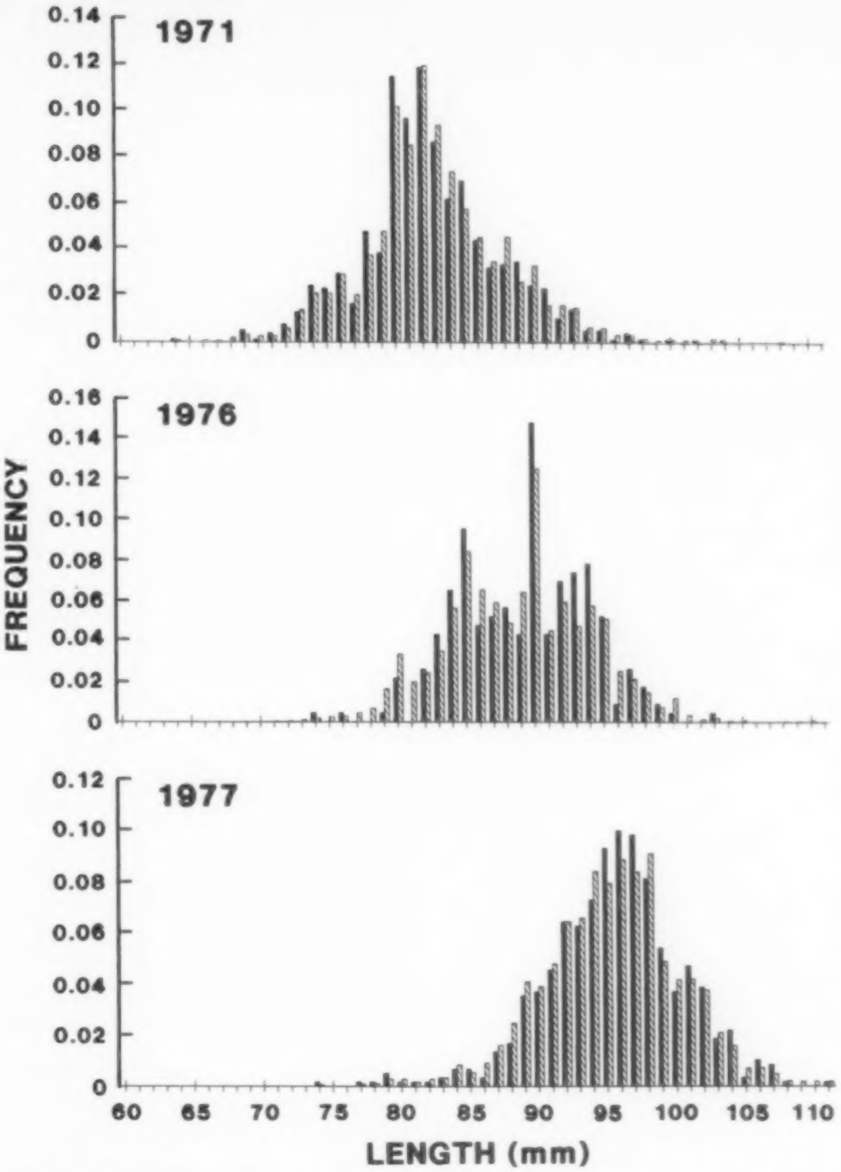

FIG. 2. Length–frequency distribution of Chilko Lake sockeye salmon smolts from the 1971, 1976, and 1977 brood years. Estimates are from daily sampling over the period of the smolt outmigration (hatched bars) and from these samples collected during the peak periods of outmigration (solid bars). See text for details.

have distinctive patterns on the freshwater portion of the scale. In particular, Chilko Lake sockeye salmon have a low circuli count compared with comigrating stocks. The total catch is apportioned among Chilko and other stocks based on these differences in the freshwater growth zone of the scale. Further, the Chilko Lake sockeye salmon stock is the largest sockeye stock during the time strata when they are present in the fishery. Generally, they represent 30–70% of the total number of sockeye taken by the fishery. As a result, small errors in the apportionment of the catch among the various stocks, based on scale patterns, would have little effect on estimates of the total catch of Chilko Lake sockeye salmon. Estimates of spawning escapement were derived using the mark–recapture method.

We used a linear model to describe the relationship between smolt-to-adult survival and smolt size:

$$A_i/S_i = a + bL_i + \sigma\epsilon_i$$

where proportional smolt-to-adult survival (A_i/S_i) is equal to the abundance of adults in the length interval i (A_i) divided by the abundance of smolts in the length interval i (S_i), b is the rate of change in survival per length increment L_i, a is the regression intercept, σ is the standard deviation of the error, and the ran-

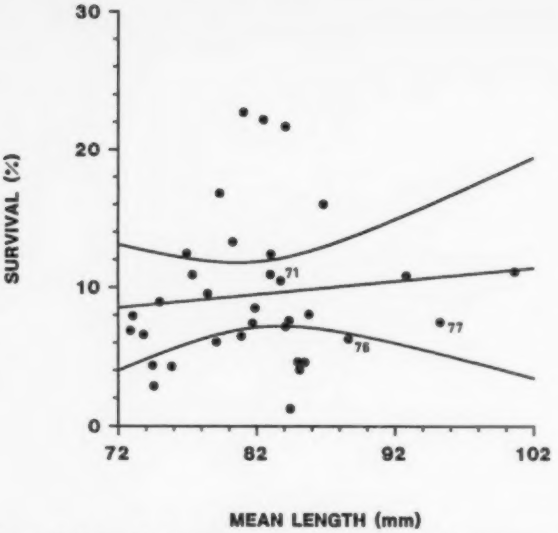

FIG. 3. Relationship between smolt-to-adult survival and mean annual smolt fork length for Chilko Lake sockeye salmon for brood years 1952–85. The 95% confidence interval is shown.

dom variables (ϵ_i) are assumed to be independent and normal with a mean of 0 and a variance of 1.

Two-tailed tests were used in all statistical analyses.

Results

Stock composition estimates based on the mixture model (Fournier et al. 1984) indicated that, for the 1971 brood year, 100% (95% confidence interval = 98–100%) of the smolts sampled for scales originated from the population at the north end of the lake. The corresponding estimate for the 1976 brood year was 96% (95% confidence interval = 90–100%). The results obtained from the mixture model for the 1971 and 1976 brood years were similar to the estimates of spawning escapement made by means of the mark–recapture method. The estimated spawning escapement of the south-end population for the 1977 brood year, the year for which we were unable to generate an estimate of smolt composition by the mixture model, was the lowest of the three years considered in our study. In this brood year, 4.3% of the total number of spawners in the Chilko Lake system were estimated to have come from the south-end population. Therefore, both the mixture model and mark–recapture approach indicate a negligible contribution of the south-end population to smolt production. Consequently, for the purpose of our analyses, we have assumed that omitting the contribution of the south-end spawning population will have no effect on the estimates of size-dependent survival based on scale samples from the north-end spawning population.

From 1952 to 1985, mean smolt fork length ranged from 72 to 101 mm and smolt-to-adult survival from about 1 to 23% (Fig. 3). However, the relationship between smolt-to-adult survival and mean smolt fork length based on this 34-yr time series gave no indication of any effect of smolt size on survival. The slope of the regression of smolt-to-adult survival on mean fork length was not significantly different from zero (*t*-test, $P > 0.05$).

Within brood years, the mean fork length of smolts contributing to adult returns, as estimated from scale characteristic R2, was greater than the mean length of smolt outmigrants for all three years examined, and the difference was significant (*t*-test) in 1971 and 1977 (Table 1). The increase in estimated mean smolt length between the smolt and the adult stage based on scale characteristic R2 suggests that smolt-to-adult survival for Chilko Lake sockeye salmon increases with increases in smolt size.

TABLE 1. Estimates of mean smolt fork length of Chilko Lake sockeye salmon for the 1971, 1976, and 1977 brood years as determined from outmigrants and adult returns. Lengths of smolt outmigrants and the size of adults as outmigrants were determined from scale characteristic R2. Sample sizes are shown in parentheses.

	Smolt fork length (mm)		
	Outmigrants	Adults	*P*
1971	81.7 (294)	82.3 (291)	<0.05
1976	88.8 (194)	89.2 (280)	>0.05
1977	95.7 (192)	97.3 (292)	<0.01

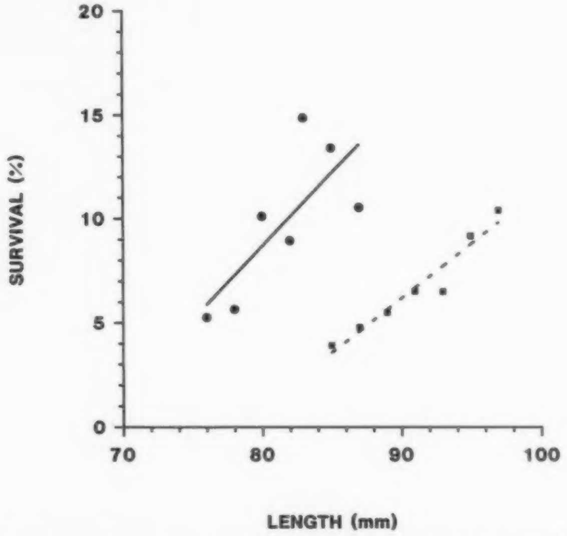

FIG. 4. Relationship between smolt-to-adult survival and fork length size-class for Chilko Lake sockeye salmon based on the 1971 brood year (●) and the 1976 and 1977 brood years (■). Size-classes are grouped into 2-mm intervals beginning with 75–77 mm.

Estimates of smolt-to-adult survival for different size-classes of smolts for the 1971, 1976, and 1977 brood years provided further evidence for size-dependent survival (Fig. 4). Regressions of smolt-to-adult survival as a function of smolt fork length were calculated separately for each brood year. There was no significant difference (*F*-test, $P > 0.05$) in the slopes of the regressions among the three years compared with a common slope, but there was a significant (*F*-test, $P < 0.05$) intercept effect. Further examination showed that there was no significant difference (*F*-test, $P > 0.05$) in the intercepts between the 1976 and 1977 brood years. Consequently, the data for the 1976 and 1977 brood years were pooled, while data for the 1971 brood year were treated separately. Smolt-to-adult survival increased with increases in the smolt fork length for the 1971 brood year (*F*-test, $P < 0.05$) and the combined 1976 and 1977 brood years (*F*-test, $P < 0.001$). An increase in smolt fork length for the 1971 brood year from 76 to 87 mm and in the 1976 and 1977 brood years from 85 to 97 mm resulted in between a two- and threefold increase in smolt-to-adult survival.

Discussion

The results of our study lead us to reject the hypothesis that smolt-to-adult survival is independent of smolt size for Chilko Lake sockeye salmon. Both analyses in our study that focused on the within brood year relationship between smolt-to-adult survival and smolt size indicated that survival increased with increases in smolt size. The mean smolt fork length based on scale characteristic R2 was significantly greater when measurements were made from adult scales than when they were made from smolt scales for two of the three brood years included in our study (Table 1). Equally convincing was the increase in smolt-to-adult survival with increases in sockeye smolt fork length based on the 1971, 1976, and 1977 brood years (Fig. 4). The increase in survival was dramatic; an approximate 14% increase in fork length resulted in a two- to threefold increase in smolt-to-adult survival for the 1971 brood year. A similar percentage increase in fork length for the 1976 and 1977 brood years combined resulted in a 175% increase in smolt-to-adult survival.

Macdonald et al. (1987) provided the only other estimates for smolt-to-adult survival as a function of smolt size-classes within a brood year for sockeye salmon. In their study, sockeye smolts of various sizes from Babine Lake, British Columbia, were marked with coded wire tags in 1967 and 1968. A portion of the adult catch and spawning escapement originating from these two smolt runs was subsequently examined for the presence of coded wire tags. The results showed an increase in the percent of tags recovered, and hence smolt-to-adult survival, with increases in the size-class of smolts. Although our results are qualitatively similar to those of Macdonald et al. (1987), the smolt-to-adult survival estimates from their study are only relative. The total adult return was not sampled for tags, nor was there an estimate of the proportion of the total return that was sampled. Further, the results from Macdonald et al. (1987) remain uncertain because of a greater but undefined level of mortality for the smaller smolt size-classes resulting directly from the coded wire tagging process and independent of any size-biased mortality occurring following the period of smolt outmigration.

Studies of two other salmonid species, steelhead trout (Ward et al. 1989) and coho salmon (Bilton et al. 1982), show a correlation between smolt-to-adult survival and smolt size within brood years. A comparison of these studies with ours, however, reveals striking qualitative and quantitative differences. Smolt-to-adult survival of steelhead trout increased with increases in the size-class of smolts. However, the rate of change in survival with change in smolt size-class was less than we observed for sockeye salmon, and the mean length and survival of the steelhead smolts were much greater. An increase in smolt-to-adult survival from approximately 5 to 15% for the 1971 brood year of Chilko Lake sockeye salmon occurred over a size range between 75 and 87 mm (Fig. 4). The 1976 and 1977 brood years combined showed an increase in smolt-to-adult survival from 4 to 11% as smolt size increased from 85 to 97 mm. A similar increase in smolt-to-adult survival of steelhead trout (4–15%) occurred over a smolt size range between approximately 150 and 170 mm. Most steelhead trout smolts were larger than 170 mm, and smolt-to-adult survival for the largest size-class, which was centered on 260 mm, approached 45%.

A more complicated pattern of smolt-to-adult survival involving variation in both sizes of smolts at release and the time of release has been shown for coho salmon (Bilton et al. 1982). Juvenile coho salmon were partioned into different size-classes and released at four different times between April 14 and July 10, 1975. Each size-class within each release time group was uniquely marked. Recovery of fish from these release groups in the commercial and recreational fisheries and the spawning escapement indicated that the greatest absolute smolt-to-adult survival came from the release of small juveniles early in the 85-d period and large juveniles toward the end of the period. Consequently, comparisons of our study with those of Ward et al. (1989) and Bilton et al. (1982) suggest that no single function can adequately describe the qualitative and quantitative relationship between smolt-to-adult survival and smolt size for all salmonids. This is probably due to differences encountered by juveniles of the three species in the marine environment resulting from differences in their timing of seaward migration, actual migration routes, and feeding regimes.

Although we found a significant increase in smolt-to-adult survival with increases in smolt size within brood years (Fig. 4; Table 1), there was no relationship between smolt-to-adult survival and mean smolt size based on pooling data from a 34-yr time series (Fig. 3). The lack of a relationship between smolt-to-adult survival and mean annual smolt size observed in our study may have been due in part to the limited variation in mean smolt size. Ricker (1962) and Koenings and Burkett (1987) combined data from several lakes and years and demonstrated a positive relationship between smolt-to-adult survival and mean smolt size for sockeye salmon. However, the range in mean smolt size observed by Ricker (1962) was from 70 to 170 mm fork length and by Koenings and Burkett (1987) was from 61 to 170 mm fork length compared with the range in our study of approximately 72–101 mm (Fig. 3).

Although our results suggest that smolt size can affect smolt-to-adult survival, smolt size is not the only, and possibly not even the most important, determinant of smolt-to-adult survival for sockeye salmon. Consequently, features other than smolt size may have obscured any relationship between smolt-to-adult survival and mean smolt size (Fig. 3). Of particular interest are those features that tend to exhibit random variation between years and hence obscure any relationship that may exist between smolt-to-adult survival and mean smolt size. Foerster (1954) examined the relationship between smolt-to-adult survival and mean smolt size for Cultus Lake sockeye salmon over an 18-yr period. Cultus Lake, like Chilko Lake, is part of the Fraser River basin. In contrast with the results of our study, Foerster (1954) found an increase in survival with increases in mean smolt size even though the range in mean smolt size in his study, 63–109 mm fork length, was similar to the range in our study. As a result, differences in the range of mean smolt size do not account for the difference in significance of the relationships between smolt-to-adult survival and mean smolt size between Foerster's (1954) study and ours. However, there are differences between the Chilko and Cultus lake systems that may explain the apparent discrepency. Cultus Lake is only 110 river km from the mouth of the Fraser River, while Chilko Lake is 725 km from the mouth. It is known that the smolt-to-adult survival of Chilko Lake sockeye salmon is positively correlated with discharge in the Fraser River and the Chilcotin River (the tributary linking Chilko Lake to the Fraser River) during the period of smolt outmigration (J. C. Woodey, Pacific Salmon Commission, Vancouver, B.C., pers. comm.). It is possible that interannual variation in features such as water discharge obscures the relationship between smolt-to-adult survival and mean smolt size for stocks such as Chilko Lake sockeye salmon that must traverse great river distances as smolts. Conversely, stocks located near the mouth of a river may not be subject to these confounding influences.

Attempts are currently being made to increase the spawning escapement of sockeye salmon to the Chilko system and other sockeye spawning areas in the Fraser River basin. The increase in the spawning escapement to the Chilko system should result in higher densities of juvenile sockeye salmon in the lake. With respect to smolt-to-adult survival, the question then becomes, what effect will the increase in juvenile density have on smolt size? Previous studies have shown that the growth of juvenile sockeye salmon in freshwater, and therefore ultimately smolt size, can either decrease with increasing juvenile density (Johnson 1965; Rogers 1980; Hyatt and Stockner 1985; Koenings and Burkett 1987; Kyle et al. 1988) or be independent of juvenile density (Koenings and Burkett 1987; Kyle et al. 1988). Furthermore, it has also been demonstrated that freshwater predation pressure caused by large juvenile sockeye salmon populations can restructure the zooplankton community of a lake (Kyle et al. 1988). This can result in a decrease in feeding efficiency and growth, not only for juveniles of large populations but also for juveniles of smaller populations in the following years. Consequently, predicting the effect of increases in the production of juvenile sockeye salmon in Chilko Lake on adult returns will require an as yet unavailable understanding of the factors controlling juvenile growth in this system.

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References

BILTON, H. T., D. F. ALDERDICE, AND J. T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–447.

CASS, A. 1989. Stock status of Fraser River sockeye salmon (*Oncorhynchus nerka*). Can. Tech. Rep. Fish. Aquat. Sci. 1674: 106 p.

CLUTTER, R. I., AND L. E. WHITESEL. 1956. Collection and interpretation of sockeye salmon scales. Int. Pac. Salmon Fish. Comm. Bull. 9: 159 p.

FOERSTER, R. E. 1954. On the relation of adult sockeye salmon (*Oncorhynchus nerka*) returns to known smolt seaward migrations. J. Fish. Res. Board Can. 11: 339–350.

FOURNIER, D. A., T. D. BEACHAM, B. E. RIDDELL, AND C. A. BUSACK. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

GOODLAD, J. C., T. W. GJERNES, AND E. L. BRANNON. 1974. Factors affecting sockeye salmon (*Oncorhynchus nerka*) growth in four lakes of the Fraser River system. J. Fish. Res. Board Can. 31: 871–892.

HAGER, R. C., AND R. E. NOBLE. 1976. Relation of size at release of hatchery-reared coho salmon to age, size and sex composition of returning adults. Prog. Fish-Cult. 38: 144–147.

HARGREAVES, N. B., AND R. J. LEBRASSEUR. 1986. Size selectivity of coho (*Oncorhynchus kisutch*) preying on juvenile chum (*O. keta*) salmon. Can. J. Fish. Aquat. Sci. 43: 581–586.

HEALEY, M. C. 1982. Timing and relative intensity of size selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. 39: 952–957.

HYATT, K. D., AND J. G. STOCKNER. 1985. Responses of sockeye salmon (*Oncorhynchus nerka*) to fertilization of British Columbia coastal lakes. Can. J. Fish. Aquat. Sci. 42: 320–331.

JOHNSON, K. A. 1970. The effect of size at release on the contribution of the 1964 brood Big Creek Hatchery coho salmon to the Pacific Coast sport and commercial fisheries. Oreg. Fish. Comm. Res. Rep. 2: 12 p.

JOHNSON, W. E. 1965. On the mechanisms of self-regulation of population abundance in *Oncorhynchus nerka*. Mitt. Int. Ver. Limnol. 13: 66–87.

KOBAYASHI, T. 1980. Salmon propagation in Japan, p. 91–109. *In* J. E. Thorpe [ed.] Salmon ranching. Academic Press, London. 441 p.

KOENINGS, J. P., AND R. D. BURKETT. 1987. Population characteristics of sockeye salmon (*Oncorhynchus nerka*) smolts relative to temperature regimes, euphotic volume, fry density and forage base within Alaskan Lakes, p. 216–234. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

KYLE, G. B., J. P. KOENINGS, AND B. M. BARRETT. 1988. Density-dependent, trophic level responses to an introduced run of sockeye salmon (*Oncorhynchus nerka*) at Fraser Lake, Kodiak Island, Alaska. Can. J. Fish. Aquat. Sci. 45: 856–867.

MACDONALD, P. D. M., H. D. SMITH, AND L. JANTZ. 1987. The utility of Babine smolt enumerations in the management of Babine and other Skeena River sockeye salmon (*Oncorhynchus nerka*) stocks, p. 280–295. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

PARKER, R. R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. 28: 1503–1510.

RICKER, W. E. 1962. Comparison of ocean growth and mortality of sockeye salmon during their last two years. J. Fish. Res. Board Can. 19: 531–547.

1982. Size and age of sockeye salmon (*Oncorhynchus nerka*) in relation to environmental factors and the fishery. Can. Tech. Rep. Fish. Aquat. Sci. 1115. 117 p.

ROGERS, D. E. 1980. Density-dependent growth of Bristol Bay sockeye salmon, p. 267–284. *In* W. J. McNeil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

STARR, P. J., A. T. CHARLES, AND M. A. HENDERSON. 1984. Reconstruction of British Columbia sockeye salmon (*Oncorhynchus nerka*) stocks: 1970–1982. Can. MS Rep. Fish. Aquat. Sci. 1780: 123 p.

TODD, I. P., AND P. A. LARKIN. 1971. Gillnet selectivity on sockeye (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) of the Skeena River system, British Columbia. J. Fish. Res. Board Can. 28: 821–842.

WARD, B. R., P. A. SLANEY, A. R. FACCHIN, AND R. W. LAND. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. Can. J. Fish. Aquat. Sci. 46: 1853–1858.

A Hydroacoustic Study of the Pelagic Spawning Behavior of Whitefish (*Coregonus lavaretus*) in Lake Constance

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Eckmann, R. 1991. A hydroacoustic study of the pelagic spawning behavior of whitefish (*Coregonus lavaretus*) in Lake Constance. Can. J. Fish. Aquat. Sci. 48: 995–1002.

The horizontal and vertical distribution patterns of pelagic spawning whitefish (*Coregonus lavaretus*) were studied by echosounding from the end of the growing season (early November) until after spawning (late January). During early November, whitefish inhabited similar depths during day and night, but during spawning displayed vertical migrations of up to 50 m amplitude: whitefish ascended at dusk and descended at dawn. At the end of the spawning season, vertical migrations ceased. Juveniles took part in the daily migrations, but only sexually mature specimens invaded the uppermost 10 m of water, while maturing and juvenile individuals and spent females stayed deeper during the night. This vertical distribution pattern is interpreted as a means to increase encounter probability among mature specimens. Diel vertical migration of the entire population is suggested to be an aid to avoid cannibalism on freshly spawned eggs during settling to the lake bottom.Total fish density estimates increased during upward migration and decreased during downward migration; this may be the result of differences in target strength caused by both reduced swimbladder volume and positive tilt while in deep waters. Highly reproducible abundance estimates were obtained during night when whitefish were evenly distributed at the top of their vertical range.

On a effectué une étude hydroacoustique des régimes de distribution horizontale et verticale du lavaret (*Coregonus lavaretus*), poisson qui fraie dans les eaux pélagiques, de la fin de la saison de croissance (début de novembre) jusqu'à la fin de la fraie (fin de janvier). Au début de novembre, le lavaret fréquentait des profondeurs semblables pendant le jour et la nuit, mais au moment de la fraie, il effectuait des migrations verticales allant jusqu'à 50 m: il remontait vers la surface au crépuscule et retournait aux eaux profondes à l'aube. À la fin de la fraie, les migrations verticales ont cessé. Seuls les individus sexuellement matures fréquentaient les premiers 10 m à partir de la surface. Les individus en phase de maturation, les juvéniles et les femelles vides restaient à de plus grandes profondeur pendant la nuit. Ce régime de distribution verticale est interprété comme un moyen d'accroître la possibilité d'interactions entre les individus matures. On formule l'hypothèse que cette migration verticale nycthémérale de toute la population est un moyen d'éviter le cannibalisme des oeufs récemment pondus pendant la phase de dépôt sur le fond. La densité totale estimative des poissons a augmenté pendant la migration vers la surface et a diminué pendant la migration vers les profondeurs. Cette variation peut être le résultat de différences de l'index de réflection des cibles acoustiques causées par le volume réduit de la vessie natatoire et l'inclinaison positive du poisson en eaux profondes. On a obtenu des estimations facilement reproductibles de l'abondance pendant la nuit lorsque les lavarets sont uniformément distribués au niveau supérieur de leur aire de répartition verticale.

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Coregonid fish can be classified into four groups according to their spawning sites: (i) littoral spawners like *Coregonus albula*, *C. clupeaformis*, *C. artedii*, and *C. pollan*; (ii) deepwater spawners like *C. hoyi*, *C. kiyi*, *C. reighardi*, and *C. pidschian* syn. *acronius* (now extinct in Lake Constance); (iii) river spawners like *Stenodus leucichthys*, *C. autumnalis*, and migratory forms of *C. oxyrhynchus* and *C. lavaretus*; and (iv) pelagic spawners like *C. lavaretus* in Lake Constance (Bodaly 1986; Dabrowski 1981; Luczynski 1986; Skurdal et al. 1985; Steinmann 1951).

The latter group is probably the least common and in fact seems to be absent from lakes in North America. In Lake Constance, however, the Blaufelchen (*C. lavaretus*, sometimes *C. l. wartmanni*) dominates in the pelagic zone and is the most important commercial species. Elster (1944) described their spawning behavior: "…maturing fish congregate between 20 and 40 m depth. As maturity progresses, males and later on females come closer to the surface. Ripe specimens remain in the uppermost ten meters of water and disperse into greater depth again after spawning." This information was obtained by gillnet and seine catches and has served as a basis for fishing regulations during spawning time. A closed season exists from October 15 until January 10. When test fishing by fishery wardens proves that at least 50% of females can be stripped, fishermen are allowed to fish, but only in the uppermost 10 m of water in order to preserve virgin and maturing specimens. The spawning fishery is closed when all hatcheries are supplied with fertilized eggs, normally within 2–4 d (Quoss 1982). Spawning usually takes place during late November and the first half of December when surface temperature has declined to about 7°C, but an influence of sunspot activity on spawning time has also been observed (Hartmann 1984). Deeper insight into the spawning behavior of Blaufelchen would be difficult to obtain by fishing techniques alone. Therefore, this study investigated temporal and spatial distribution patterns of Blaufelchen before, during, and after spawning time by acoustic methods.

Materials and Methods

Study Site and Species Studied

Lake Constance (Bodensee) is the second largest (to Lake Geneva) European prealpine lake and the largest lake in Germany. Its main basin ("upper lake") has a surface area of 476 km^2, a volume of 47.7 km^3, a maximum depth of 252 m, and a mean depth of 100 m (Geller and Guede 1989). The lake is warm-monomictic and has generally no ice cover in winter. Acoustic surveys can therefore be undertaken all year.

The fish fauna is composed of about 30 species, but whitefish (*C. lavaretus*) and perch (*Perca fluviatilis*) are by far the most important economically. Whitefish are present in two ecologically distinct forms in the upper lake: Gangfisch, which spawn in shallow to medium-deep littoral waters, and Blaufelchen, which spawn close to the surface in the pelagic zone over water depths of up to 250 m (Eckmann 1987; Roesch 1987; Ruhlé 1986; Vuorinen et al. 1986). During the growing season, both forms probably cooccur as pelagic planktivores, but around spawning time they segregate according to their spawning sites. During spawning time, the pelagic fish community comprises Blaufelchen almost exclusively, with very little bycatch of trout and char. This situation allows the assumption that all the data obtained by hydroacoustics in the pelagic zone apply to Blaufelchen.

Fish were sampled with two fleets of monofilament gill nets of 25-, 32-, 38-, 42-, and 44-mm bar mesh during the night of December 12 over approximately 100 m water depth. The first fleet (160 m long) was hung by 10-m droplines from surface floats and thus fished roughly from 10 to 17 m depth, while the second fleet (120 m long) fished from about 27 to 34 m. Nets were exposed after dusk, when the fish had achieved their nighttime vertical distribution. They were taken in some 4–5 h later. Additionally, a 4-m-wide by 2-m-high trawl was used. It was pulled at 1 m·s^{-1} at different depths. Temperature profiles were taken with a self-assembled temperature/depth probe. Light intensity was measured with a luxmeter B350D (LMT, Berlin), measuring range 1×10^{-3} to 2×10^4 lx.

Hydroacoustic Methods

A single-beam echosounder, Simrad EY-M, was used. Its working frequency was 70 kHz, and ping rates were 182 and 91·min^{-1} in the 60- and 120-m range, respectively. Time-varied gain (TVG) was 40 log *R*, compensating for spherical spreading and absorption of the acoustic energy to 60 m. All echoes received from depths greater than 60 m were numerically corrected during data processing. The TVG function begins at 2 m, so with the mounting of the transducer (see below), fishes in the upper 3 m will be excluded.

The echosounder has a calibrated output, where the analog signals, having been shifted down to 10 kHz, can be recorded on an ordinary high-fidelity tape recorder. A Sony TC-D5M was used; dynamic range of the tapes was 52 dB at 10 kHz. At the start of each tape, the internal calibration signal was recorded to allow reproducible setting of the AD converter. A system calibration was realized with a standard copper sphere. During the first surveys (1987), however, a different transducer had been used, which had only been calibrated with ping-pong balls. The new transducer was therefore calibrated with both copper sphere and the identical ping-pong balls from the 1987 surveys. The difference between both calibration procedures was only 0.7 dB but the former calibration was corrected accordingly. Data tapes were digitized, the current envelope being sampled every 0.1 ms, and all information including ping number, depth, echo amplitude, and water depth was stored on hard disc in a personal computer. A program was developed which allows reconstruction of echograms on a plotter.

The transducer has a circular beam pattern whose beamwidth at the −3 dB level was found to be 11.2° (data provided by the manufacturer). This value was substituted in the theoretical directivity function of a circular transducer and its effective diameter calculated. The directivity pattern was then established and the beam split into 2-dB classes. For the present study, ten 2-dB classes from −32 to −52 dB were considered, and all echoes stronger than −32 dB were shifted down into the −33/−34 dB class. Working with the 40 log *R* TVG function, the echo amplitude of single fish echoes depends only on the angular coordinates of the fish. The target strength (TS) distribution was obtained by using the Craig and Forbes (1969) method to remove the effect of the beam pattern from the distribution of the echo amplitudes (cf. Lindem 1983).

When analyzing the data tapes, single-fish echoes are sorted out, taking into account possible vertical and horizontal overlap of echosignals. Furthermore, the energy found in both single and multiple echoes is integrated. By assuming that the TS distribution of the single fish is representative of all fish, the total fish density is estimated. The water column is divided into 10- or 20-m depth layers, according to the number of single-fish echoes received. When the number of single-fish echoes in the uppermost layer (from 3 to 10 m depth) is less than 10, the calculated fish density is considered unreliable and is excluded from further calculations. TS (decibels) was transformed into fish length (*L*, centimetres) according to a relationship established by Borisenko et al. (1989) for whitefish from 100 to 1300 g body mass: $TS = 20.97 \log L - 66.4$

To estimate the vertical distribution for different size groups of whitefish, the ten 2-dB classes were pooled into three groups: less than −32 to −36 dB, less than −36 dB to −40 dB, and less than −40 dB to −52 dB. These correspond to total fish lengths of 43–28, 28–18, and 18–5 cm, respectively. The quartile depths for these three TS groups were estimated from their cumulative percentage distributions versus depth, which were assessed in 2-m steps of depth increase. In the deconvolution technique according to the Craig and Forbes (1969) method, low numbers and/or irregular vertical distribution of the strongest echoes will lead to imprecise estimates of quartile depths for all three TS groups. Therefore, only those transects were considered which contained more than 20 echoes in the less than −32 dB to −36 dB group. The central part of Lake Constance (between Nonnenhorn, Rohrschach, Friedrichshafen, and Romanshorn) is known to be the main spawning area of Blaufelchen (Fig. 1). Acoustic data sampled along transects (Fig. 1) demonstrated that, from November through January, fish densities were always highest towards the northeastern shore. For this reason, vertical distribution patterns were mainly studied in front of Langenargen. The transducer was mounted in a fin and suspended about 0.75 m from the side of the boat at a depth of about 1 m. Boat speed during all surveys was about 2.3 m·s^{-1}. For the present study, 16 h of tape recordings, consisting of 29 day and 26 night transects, were considered (Table 1).

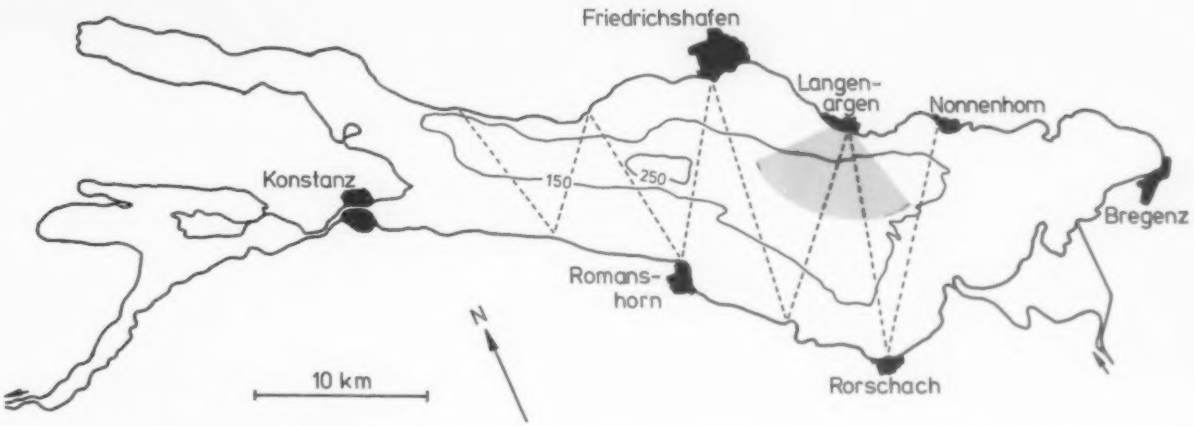

FIG. 1. Map of Lake Constance showing the transects for acoustic surveys on horizontal fish distribution (broken lines) and the survey area where vertical migration was studied (shaded area).

TABLE 1. Dates of acoustic surveys during spawning time in 1989–90, number of transects which were completed before or after the onset of darkness (day/night), total number of analyzed pings (excluding all pings that were overshadowed by "ghost-bottom" echoes), total time covered by analyzed pings, and total number of single-fish echoes.

Date	Day/ night	Analyzed pings	Time (min)	Single-fish echoes
Nov. 8	1/2	16 672	91	12 774
Nov. 23	4/3	11 894	105	6 354
Nov. 25	3/9	14 425	122	8 965
Nov. 29	4/2	14 304	123	7 413
Dec. 1	4/2	17 271	141	3 656
Dec. 10	2/1	8 033	83	4 715
Dec. 12	4/1	11 236	103	3 339
Dec. 21	2/3	7 562	51	2 600
Dec. 27	3/1	11 055	65	8 375
Jan. 23	2/2	11 522	77	18 702
Total	29/26	123 974	961	76 893

Results

At the end of the growing season, Blaufelchen occupied similar depths during day and night. Most fish were found from 10 to 30 m (Fig. 2, November 8). This pattern changed markedly toward spawning time. Blaufelchen then were found at considerably greater depth during the day, down to 100 m or even deeper. Their nighttime vertical distribution, however, was similar to that in early November (Fig. 2, November 23). During spawning, which took place around December 10 in 1989, the nighttime vertical depth range was broader, reaching from the water surface to 40 m depth. After spawning (Fig. 2, December 27), the difference between day and night distribution gradually disappeared. By January 23, the fish again occupied very similar depths during day and night, but their depths then extended over a wider depth range and were deeper (from 20 to 60 m) than at the end of the growing season (Fig. 2, cf. November 8 and January 23).

During spawning, fish ascended during dusk and descended at dawn (Fig. 3). During daylight hours, pelagic whitefish were distributed from 20 to 100 m. As light intensity decreased, fish gradually concentrated in the upper layers until none were observed below 60 m at the onset of darkness (defined as $<10^{-2}$ lx 2 m above the water surface). During the present study, darkness started around 17:30 and ended around 07:00. After the onset of darkness, few echoes were received from the 40- to 60-m layer, so the population stayed in the water column from the surface to 40 m during the night. Approximately 90% of the population was concentrated in the uppermost 30 m of water (Fig. 3). At dawn, fish left the upper layers and the first echoes from the 80- to 100-m layer were recorded 1 h after darkness. The typical morning distribution was established within about 2 h (Fig. 3). Two hours before the onset of darkness, on the other hand, fish were already concentrated at much shallower depth. The speed of vertical migration was thus higher at dawn than at dusk. This behavior seems to be independent of light conditions because light intensities were similar for the two situations considered here (5×10^3 to 2.5×10^3 versus 4×10^3 lx). Additionally, the same pattern was observed on different dates and under different weather conditions during the spawning seasons in 1987 and 1989.

Total fish density estimates fluctuated in a reproducible fashion during diel vertical migration, increasing during dusk and decreasing during dawn (Table 2). In parallel, the percentage of fishes with TS less than −40 dB was highest when fish densities were lowest. The most similar estimates of total fish density were obtained during nighttime hours (Table 2). Each of the last two estimates on November 29 and of the first two estimates on December 1, respectively, was made on the same transect, first from inshore to offshore waters and then vice versa. The overall density estimates differed by only 1.1 and 4.0% of the means, respectively. Furthermore, the composition of the population by the three TS groups considered here was also very similar among pairwise recordings (Table 2). Independent estimates on different days, however, did not agree as well because horizontal distribution of Blaufelchen varied within the study area. Pelagic fish were most concentrated in a band which stretched from shallow littoral waters to approximately 160 m water depth. Towards the center of the lake, fish densities decreased. Additionally, other echo traces (probably from perch) appeared on the records between 30 and 50 m depth and extended from the sloping bottom towards the pelagic zone. To exclude fish other than pelagic whitefish, echograms were only evaluated offshore from the 120-m depth contour in this

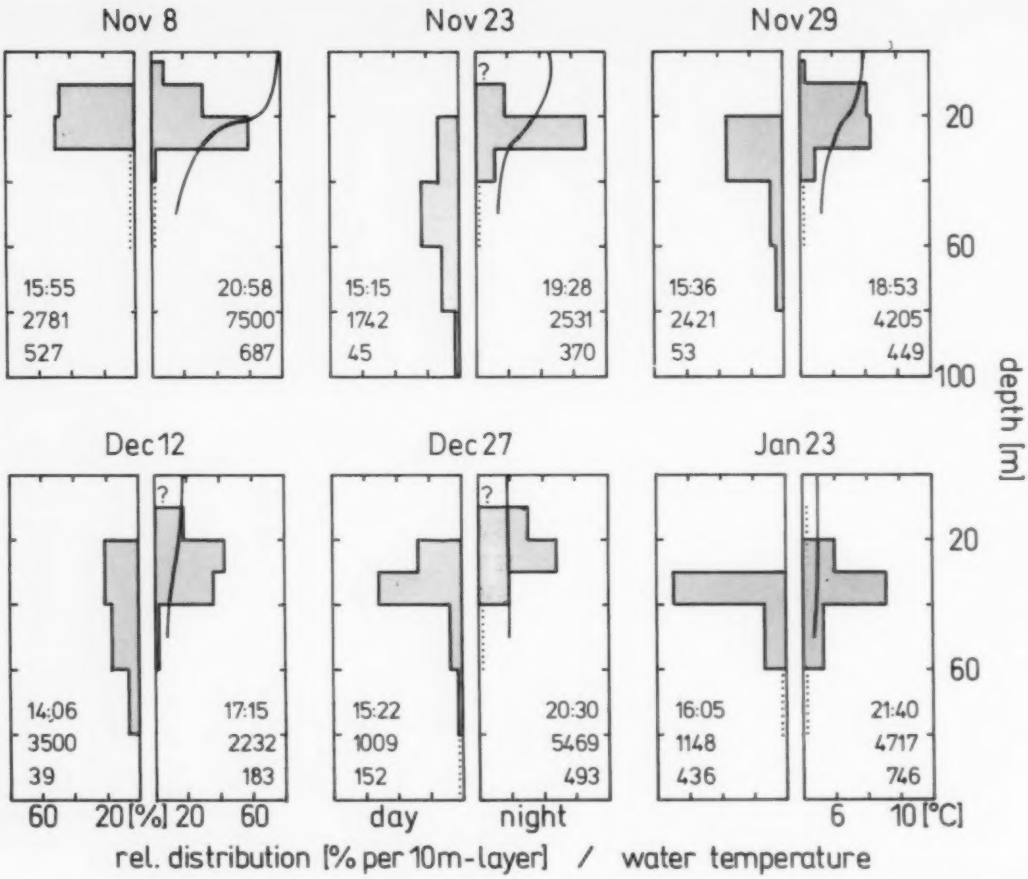

FIG. 2. Day and night vertical distribution of pelagic whitefish in front of Langenargen for six dates during the spawning season in 1989–90. Percentage distributions of the fish population per 10-m layers are shown as histograms. Dotted vertical lines: low percentage values which cannot be graphed properly. Question mark: density estimate uncertain in this depth layer due to low number of single-fish echoes. Temperature profiles are plotted as solid lines over the nighttime histograms, irrespective of the time when they were sampled. Numbers below the histograms indicate from top to bottom median time (CET) of the acoustic survey, number of analyzed pings, and estimated fish density (ha^{-1}).

study. However, no offshore limit was considered, and therefore, fish density estimates varied with the proportion of the dense fish band within each transect. Among the different recordings made on one particular day, which served as a basis for the analyses of vertical migration, the transects were kept as constant as possible. Between days, however, the transects varied with wind direction. This resulted in density estimates for the survey area which range from 222 to 640 (mean 392 ± 55, $n = 7$) fish·ha^{-1} for the time from November 23 to December 27.

Gillnet catches showed that most of the males in both depth layers (10–17 and 27–34 m) were mature or ripe and running on December 12, and the few spent males (10 out of 122 in total) were mainly found at greater depth (Fig. 4). For the females, the pattern seems to be even more clear-cut, although only eight females were caught in the lower fleet: spent females aggregate in deeper waters. They seem to do so within a short time after having shed their eggs because spawning had just started some days ago, and yet ripe and spent females were already efficiently separated from each other.

The analysis of TS distribution for the pelagic fish indicated that, apart from mature specimens, young age-classes also took part in diel vertical migrations. The presence of 0+ fish was confirmed by 100 min of trawling on the night of December 12, which caught eight Blaufelchen from 11 to 14 cm total length at a trawling depth of about 30 m.

Population gravity centers (PGCs) were similar for the three size groups both before (November 8) and after (January 23) the spawning season (Fig. 5). During spawning time, however, they differed markedly. The bigger fishes were always found in shallower water. In parallel, the vertical distribution ranges of all three size groups increased after the end of the growing season, but they did not decrease again after the end of the spawning time.

Discussion

The analysis of single-beam echosounder data by the Craig and Forbes (1969) method will provide reliable fish density

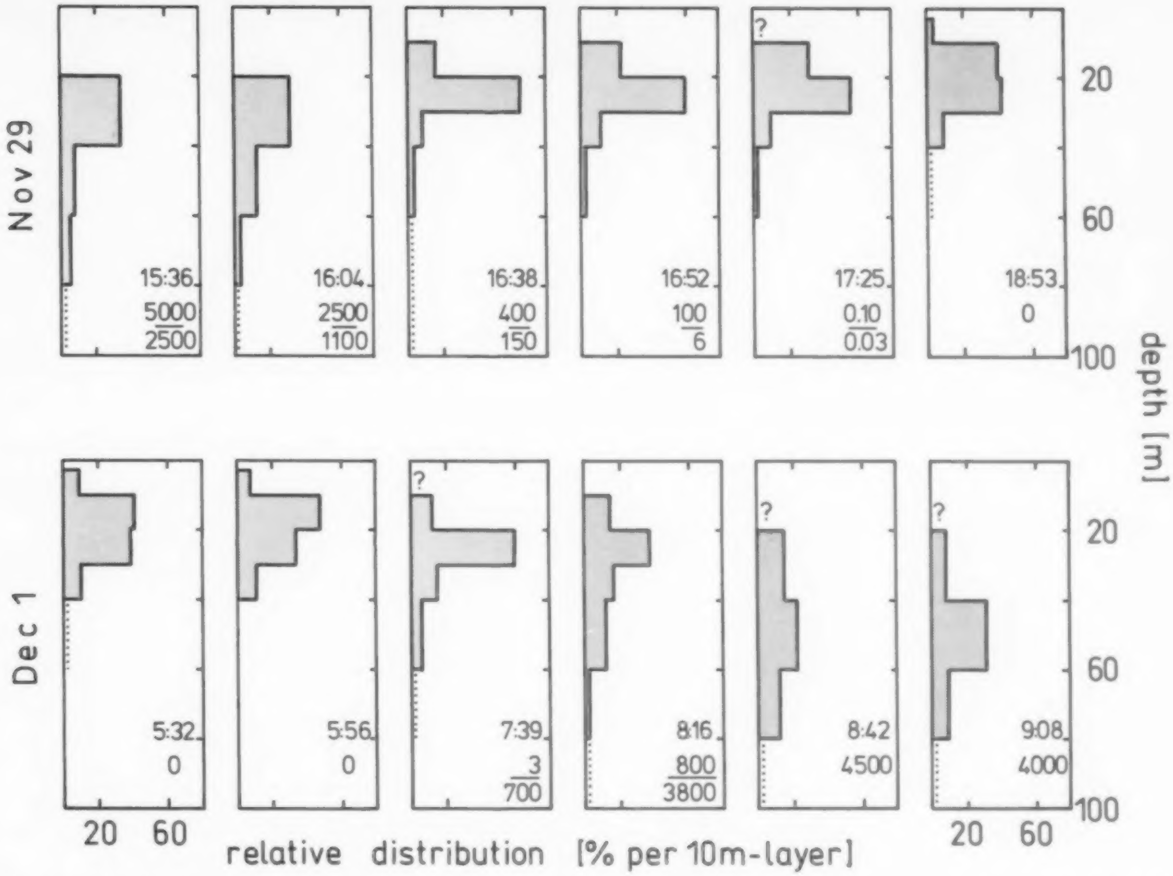

FIG. 3. Vertical migration of pelagic whitefish during dusk (upper panels) and dawn (lower panels). Histograms are as in Fig. 2. Numbers below histograms indicate median time (CET) of acoustic sampling and light intensity (lx) above the lake surface during each transect.

TABLE 2. Changes in total fish density estimates during dusk (November 29) and dawn (December 1) vertical migrations. Given for each transect are time from start to end, light intensity, total number of analyzed pings, number of single-fish echoes, percentage of total echo energy which was attributed to fish schools, total fish density, and estimated percentage composition by number of the population, divided into three size groups (from left to right): large fish (less than −32 to −36 dB), medium-sized fish (less than −36 to −40 dB), and small fish (less than −40 to −52 dB).

Time (CET)	Light intensity (lx)	Pings	Single-fish echoes	Schools (%)	Density (no·ha^{-1})	Size composition (%)		
Dusk (Nov. 29)								
15:16–15:55	$5 \times 10^3 - 2.5 \times 10^3$	2421	414	51	53	14	13	73
15:55–16:14	$2.5 \times 10^3 - 1.1 \times 10^3$	1521	349	51	64	17	11	72
16:33–16:43	400–150	886	366	40	224	11	29	72
16:44–17:01	100–6	3018	1184	31	193	23	19	58
17:19–17:31	0.1–0.03	2253	1867	36	444	28	19	53
18:40–19:06	0	4205	3233	30	449	20	28	52
Dawn (Dec. 1)								
05:27–05:38	0	1913	668	32	231	21	22	57
05:50–06:02	0	2179	636	32	222	27	12	61
07:20–07:58	3–700	6335	1387	62	164	6	26	68
08:00–08:31	$0.8 \times 10^3 - 3.8 \times 10^3$	2812	423	68	99	15	3	82
08:37–08:47	4.5×10^3	644	146	72	98	2	1	97
08:48–09:27	4.0×10^3	3388	396	75	50	1	14	85

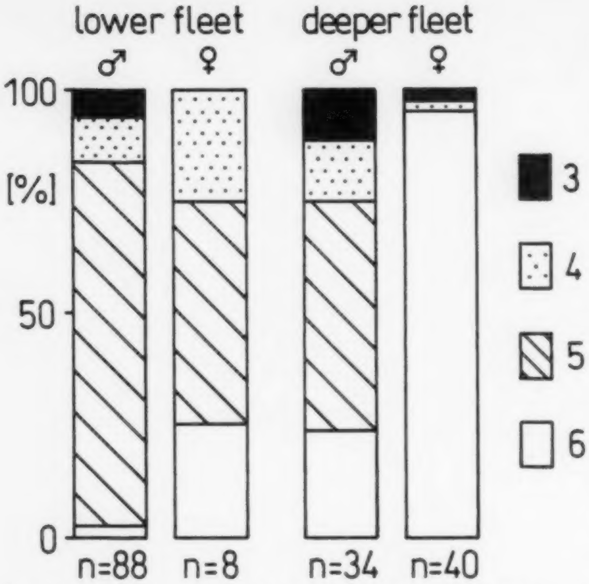

FIG. 4. Percentage composition by maturity stages, divided by sexes, for pelagic whitefish sampled from two different depth layers on December 12, 1989. Maturity stages according to Nikolsky.

estimates if the number of single-fish echoes is sufficiently high (>1000) (Jurvelius and Heikkinen 1988; Jurvelius et al. 1988; Lindem 1983). This was not always the case in the present study (Tables 1 and 2) because (i) transects of short duration are optimum for following rapid vertical migrations and (ii) in many recordings, "ghost-bottom echoes" overshadowed fish recordings, so the final number of analyzed pings was lower than the number of recorded pings. However, the analyses always revealed very consistent distribution patterns, and the reproducibility among successive recordings on the same transects was extremely high (Table 2).

The observed trend in total fish density estimates (Table 2) could be the result of regular horizontal migrations between littoral and pelagic waters like those described for yellow perch (*Perca flavescens*) and cisco (*C. artedii*) (Engel and Magnuson 1976) and for cyprinids (Bohl 1980). Yet, the present data suggest that horizontal migration is irrelevant in the case of Blaufelchen. Firstly, there is no indication that fish densities in the littoral zone increase during the day. By contrast, there is a similar vertical migration, although of less amplitude, of fish in littoral waters, including similar fluctuations of overall fish density during dawn and dusk. Secondly, diel horizontal migrations have mostly been described for small lakes of a few hundred metres in diameter. The changes in total fish density reported here, however, were recorded between 1 and 4 km offshore. Although Blaufelchen of Lake Constance may undergo some kind of horizontal movement during spawning, the systematic decrease and increase of their total density in pelagic waters during a 24-h period is most likely due to some other mechanism.

The gas-filled swimbladder of bony fish is responsible for returning up to 95% of the echo signal (Foote 1980). Any change in swimbladder volume will therefore cause a corresponding TS shift. The swimbladder volume is normally adjusted to give fish neutral buoyancy at their living depth

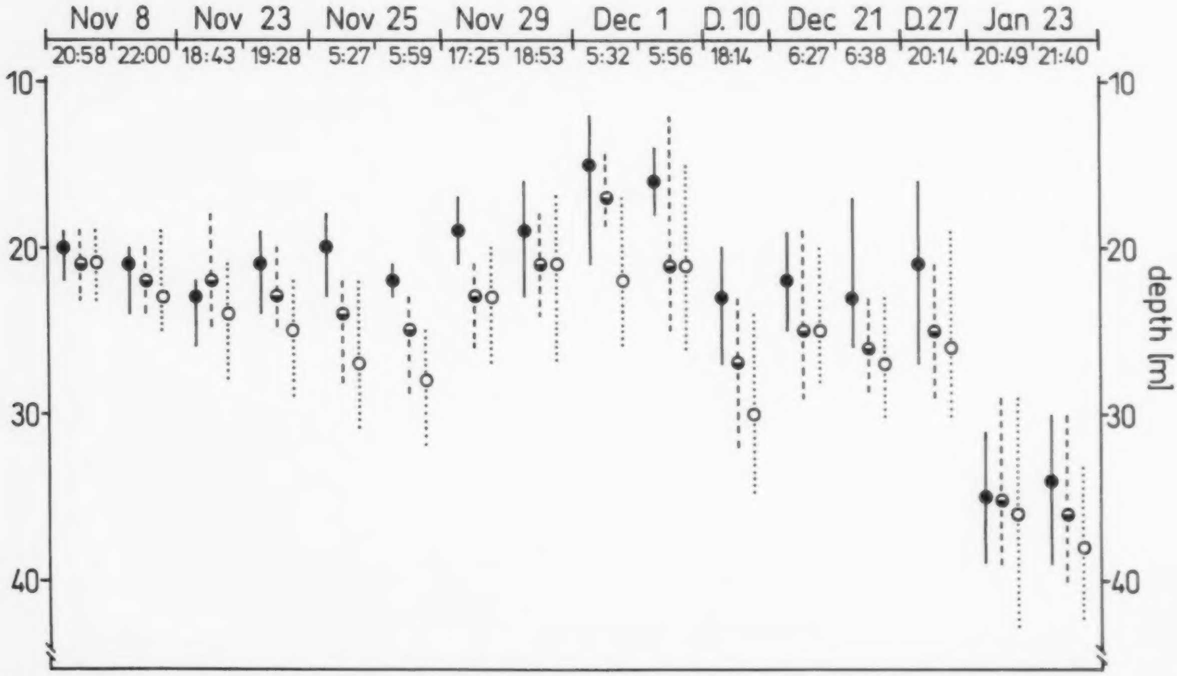

FIG. 5. Population gravity centers (PGCs) and upper (Q_{25}) and lower (Q_{75}) quartile depths during nighttime hours for three size groups of pelagic whitefish during the spawning season in 1989–90. PGCs are indicated by circles and Q_{25} and Q_{75} by the upper and lower ends of the vertical bars, respectively. Large fish (less than −32 to −36 dB): solid circles and lines; medium-sized fish (less than −36 to −40 dB): half-filled circles and broken lines; small fish (less than −40 to −52 dB): open circles and dotted lines. Median time of data sampling is indicated.

(Alexander 1966; Marshall 1972). When fish change their living depth in days or weeks, they are able to adjust the pressure in their swimbladder accordingly, so that neutral buoyancy is maintained. In the case of diel vertical migration, the situation is different: whatever might be the gas secreting mechanism, it seems to be too slow to permit compensation for rapid depth changes (Alexander 1972; Blaxter and Tytler 1978; Harden Jones and Scholes 1985; Wittenberg 1958). Therefore, the swimbladder volume of Blaufelchen must change considerably during their extensive diel vertical migrations, decreasing during the morning downward migration. In most teleosts, the swimbladder behaves like a gas bubble over a wide range of pressures, and thus, its volume can be estimated from Boyle's Law (Alexander 1966). If a whitefish is assumed to live at the uppermost limit of the population's vertical range during both day (20 m) and night (0 m), then its swimbladder volume will be reduced to about 33% of its original volume during the day. For a fish which stays at the lowermost limit of the population's vertical range (40–100 m), the corresponding value is 45%.

In order not to sink, the fish must compensate for their negative buoyancy by producing hydrodynamic lift through higher swimming speed, increased pectoral fin extension, positive tilt of the body, or by a combination of these measures. For Atlantic cod (*Gadus morhua*), it was found that a reduction of swimbladder volume by 55–90% can be compensated for by fin strokes and/or pectoral fin extension alone (Harden Jones and Scholes 1985; Ona 1990), while herring in addition showed higher swimming speed and positive tilt when lowered from the surface to 30 m depth (Ona 1990). No similar experiments have been reported for whitefish. However, a preliminary test in which several whitefish of 20 to 25 cm total length were exposed to pressures of 2 or 3 atm (1 atm = 101.325 kPa) showed that the fish either rested on the bottom of the pressure tank or they swam with remarkably strong tail beat and considerable positive tilt. Their swimming speed was clearly constrained by the size of the tank, so that they had no possibility for producing hydrodynamic lift by increased fin extension and higher swimming speed. However, I believe that even under free swimming conditions they will show some positive tilt.

Apart from the reduction of swimbladder volume during downward migration, a positive tilt of the fish body will also lead to a significant reduction of TS. Both the swimbladder and the fish body itself have a directional pattern of sound reflection, maximum sound energy being reflected roughly perpendicular to the fish's longitudinal axis. The stronger the fish's tilt, the smaller will be the echo intensity reflected towards the transducer. The systematic decrease and increase of total fish density, together with a TS shift towards lower and higher values, is, therefore, interpreted as the result of uncompensated downward and upward migrations, respectively. The unexplained lower density of vendace (*C. albula*) during daytime in Lake Paasivesi (Jurvelius and Heikkinen 1988) is probably due to the same phenomenon.

From the preceding arguments, together with certain limitations of acoustic techniques in general, it follows that the vertical migration amplitudes shown in Fig. 2 and 3 are only minimum estimates. The real fish density in the upper 10 m of water during nighttime is systematically underestimated because (i) fishes within the uppermost 3 m are totally excluded from acoustic sampling, (ii) those which stay within the next few metres are probably frightened away by boat noise, and (iii) all single-fish echoes from the 3- to 10-m layer which did not number more than 10 were excluded from density estimates as a precaution. The daytime distribution pattern, on the other hand, is systematically biased against the detection of fish in deeper waters, because increasingly smaller proportions of the population are detected with increasing depth beyond 60 m. Improved techniques will provide increased estimates of both migration amplitudes and vertical distribution ranges. The problem of TS fluctuations with diel vertical migration, however, can only be solved by continuous monitoring of in situ TS (Ona 1990).

Pelagic whitefish of Lake Constance have long been known to realize daily and seasonal vertical migrations (Elster 1944). Extensive daily vertical migrations during spawning time, however, have never been documented. These migrations occur only during the spawning season, from about 6 wk before until 2 wk after peak spawning activity. They should, therefore, bear a special importance for whitefish reproductive biology. For vendace in Lake Pluszne, a kind of vertical spawning migration has been described, too, but this lake is only about 40 m deep and migration amplitudes are much smaller than those reported here (Dembinski 1971).

Regular movements towards, and away from the spawning ground, in the case of Blaufelchen the upper metres of the water column, might help to synchronize spawning activity within a population. Littoral spawning whitefish, on the other hand, seem not to depend on similarly extensive movements to synchronize their spawning behavior. Both groups of whitefish, however, show specific migration patterns which seem to be appropriate to assure reproductive success. Littoral spawners migrate to spawning areas in late fall. Males generally arrive earlier at spawning grounds and remain there for some time, while females visit spawning grounds only to deposit their eggs and then retire to deeper waters (Dabrowski 1981; Luczynski 1986).

Blaufelchen behave in much the same way. Males arrive earliest in the upper metres of water and predominate by numbers in surface samples throughout the spawning season. Females come to surface waters only when they are ripe or when they are close to being mature (Elster 1944). This behavior clearly increases encounter probability among mature specimens, an obvious necessity in a largely homogeneous environment like the pelagic zone of a large lake. Vertical separation of size groups (Fig. 5), detected only during spawning time, and the fast retreat of spent females into deeper waters (Fig. 4) can also be interpreted in this way.

Encounter probability for mature specimens is thus increased by the fishes' distribution patterns during nighttime. However, I believe that the ultimate contact between males and females is established at dawn, and that spawning takes place during daytime hours. This reasoning is based on repeated acoustic observations of the schooling behavior of Blaufelchen. During spawning time as well as during the growing season, Blaufelchen are always found in schools during the day. Schools break up in the evening and fish are fairly evenly distributed during the night. There is no evidence to suggest that school formation in whitefish is possible by other than visual stimuli. The same will no doubt be true for the contact among mature specimens. Unfortunately, traditional acoustic techniques with a transducer towed near the surface cannot elucidate this phase of the reproductive behavior any further. Elster's (1933) article contains the only published observation on the spawning act of Blaufelchen, which took place close to the water surface during daylight hours.

If it were the only problem in spawning behavior of Blaufelchen to ensure encounter of mature individuals, it would still be difficult to understand why the whole population realizes such extensive daily migrations. I suggest that the importance of this behavior lies in reducing the risk of cannibalism on freshly spawned eggs. There is no a priori reason to preclude the possibility that whitefish will eat their own eggs. Firstly, Skurdal et al. (1985) documented egg cannibalism in a river-spawning whitefish population. Secondly, freshly spawned as well as water-hardened whitefish eggs are eaten by both immature and mature whitefish kep in aquaria and tanks. And thirdly, 1 out of 38 spent females which had been caught in the lower gillnet fleet contained 12 whitefish eggs in its stomach.

Freshly spawned whitefish eggs sink to the bottom at a rate of about 1 $m \cdot min^{-1}$ at 4°C (Elster 1944). They would thus be exposed for 30 min to potential predators if Blaufelchen would maintain their nighttime distribution (90% of the population above 30 m) during the day as well. Typical light thresholds for feeding are in the order of 10^{-2} to 10^{-3} lx for larvae and juvenile whitefish (Dabrowski 1982; Keller 1984; Volkova 1973). However, with increasing depth, foraging ability of visually oriented predators like whitefish is clearly constrained by light conditions (10^{-2} to 10^{-3} lx observed at 50–60 m depth in Lake Constance during early winter). Therefore, the risk of egg cannibalism will be increasingly lower the deeper the fish descend during the day. As soon as they ascend to lower depth in the afternoon, conditions for visual foraging improve. The fishes' residence time in depth strata of more favorable light conditions is significantly longer in the afternoon than in the morning (cf. Fig. 3). Therefore, I suggest that feeding is most intense in the afternoon. According to the reasoning about the adaptive significance of diel vertical spawning migration, spawning activity should be distinctly lower or may be even nil at that time of the day, a hypothesis which is untested so far.

Acknowledgements

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References

ALEXANDER, R. MCN. 1966. Physical aspects of swimbladder function. Biol. Rev. 41: 141–176.

1972. The energetics of vertical migration by fishes. Symp. Soc. Exp. Biol. 26: 273–294.

BLAXTER, J. H. S., AND P. TYTLER. 1978. Physiology and function of the swimbladder. Adv. Comp. Physiol. Biochem. 7: 311–367.

BODALY, R. A. 1986. Biology, exploitation and culture of coregonid fishes in Canada. Arch. Hydrobiol. Suppl. Ergebn. Limnol. 22: 1–30.

BOHL, E. 1980. Diel pattern of pelagic distribution and feeding in planctivorous fish. Oecologia 44: 368–375.

BORISENKO, E. S., A. G. GUSAR, AND S. M. GONCHAROV. 1989. The target strength dependence of some freshwater species on their length–weight characteristics. Proc. Inst. Acoust. 11(3): 27–34.

CRAIG, R. E, AND S. T. FORBES. 1969. Design of a sonar for fish counting. Fisker. Dir. Skr. Ser. HavUnders. 15: 210–219.

DABROWSKI, K. R. 1981. The spawning and early life history of the pollan (*Coregonus pollan* Thompson) in Lough Neagh, Northern Ireland. Int. Rev. Gesamten Hydrobiol. 66: 299–326.

1982. The influence of light on feeding of fish larvae and fry *Coregonus pollan* (Thompson) and *Esox lucius* (L.). Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere 86: 341–351.

DEMBINSKI, W. 1971. Vertical distribution of vendace *Coregonus albula* L. and other pelagic fish species in some Polish lakes. J. Fish Biol. 3: 341–357.

ECKMANN, R. 1987. A comparative study on the temperature dependance of embryogenesis in three coregonids (*Coregonus* spp.) from Lake Constance. Schweiz. Z. Hydrol. 49: 353–362.

ELSTER, H.-J. 1933. Beitraege zur Biologie des Blaufelchens (*Coregonus wartmanni* Bloch). Int. Rev. Gesamten Hydrobiol. 30: 181–246.

1944. Ueber das Verhaeltnis von Produktion, Bestand, Befischung und Ertrag sowie ueber die Moeglichkeit einer Steigerung der Ertraege, untersucht am Beispiel der Blaufelchenfischerei des Bodensees. Z. Fisch. 42: 169–357.

ENGEL, S., AND J. J. MAGNUSON. 1976. Vertical and horizontal distribution of coho salmon (*Oncorhynchus kisutch*), yellow perch (*Perca flavescens*), and cisco (*Coregonus artedii*) in Pallette Lake, Wisconsin. J. Fish. Res. Board Can. 33: 2710–2715.

FOOTE, K. G. 1980. Importance of the swimbladder in acoustic scattering by fish: a comparison of gadoid and mackerel target strengths. J. Acoust. Soc. Am. 67: 2084–2089.

GELLER, W., AND H. GUEDE [ED.] 1989. Lake Constance — the largest German lake, 9–17. *In* W. Lampert and K.-O. Rothhpaut [ed.] Limnology in the Federal Republic of Germany. International Association for Theoretical and Applied Limnology, Ploen 1989.

HARDEN JONES, F. R., AND P. SCHOLES. 1985. Gas secretion and resorption in the swimbladder of the cod *Gadus morhua*. J. Comp. Physiol. 155: 319–331.

HARTMANN, J. 1984. 11-year cycle of spawning time and growth of the whitefish *Coregonus lavaretus* of Lake Constance, Western Europe. Schweiz. Z. Hydrol. 46: 163–170.

JURVELIUS, J., AND T. HEIKKINEN. 1988. Seasonal migration of vendace, *Coregonus albula* (L.), in a deep Finnish lake. Finn. Fish. Res. 9: 205–212.

JURVELIUS, J., T. LINDEM, AND T. HEIKKINEN. 1988. The size of a vendace, *Coregonus albula* L., stock in a deep lake basin monitored by hydroacoustic methods. J. Fish Bio. 32: 679–687.

KELLER, T. 1984. Lichtgesteuerte Nahrungsaufnahme bei Felchen. Diploma thesis, University of Constance, Konstanz, FRG. 47 p.

LINDEM, T. 1983. Success with conventional in situ determination of fish target strength. FAO Fish. Rep. 300: 104–111.

LUCZYNSKI, M. 1986. Review on the biology, exploitation, rearing and management of coregonid fishes in Poland. Arch. Hydrobiol. Suppl. Ergebn. Limnol. 22: 115–140.

MARSHALL, N. B. 1972. Swimbladder organization and depth ranges of deep-sea teleosts. Symp. Soc. Exp. Biol. 26: 261–272.

ONA, E. 1990. Physiological factors causing natural variations in acoustic target strength of fish. J. Mar. Biol. Assoc. U.K. 70: 107–127.

QUOSS, H. 1982. Zum Blaufelchenlaichfischfang am Bodensee-Obersee. Fischwirt 32: 1–3.

ROESCH, R. 1987. Egg-size of pelagic and nearshore spawning coregonids (*Coregonus lavaretus* L.) from Lake Constance correlated with the fish weight. Arch. Hydrobiol. 109: 519–523.

RUHLÉ, C. 1986. Contribution to the problem of differentiation of "Blaufelchen" (*Coregonus lavaretus wartmanni* L.) and "Gangfisch" (*Coregonus lavaretus macrophthalmus* L.) in Lake Constance. Arch. Hydrobiol. Suppl. Ergebn. Limnol. 22: 283–289.

SKURDAL, J., E. BLEKEN, AND N. C. STENSETH. 1985. Cannibalism in whitefish (*Coregonus lavaretus*). Oecologia 67: 566–571.

STEINMANN, P. 1951. Monographie der schweizerischen Coregonen. Schweiz. Z. Hydrol. 13: 54–155.

VOLKOVA, L. A. 1973. The effect of light intensity on the availability of food organisms to some Lake Baikal fishes. J. Ichthyol. 13: 709–722.

VUORINEN, J., A. CHAMPIGNEULLE, K. DABROWSKI, R. ECKMANN, AND R. ROESCH. 1986. Electrophoretic variation in central European coregonid populations. Arch. Hydrobiol. Suppl. Ergebn. Limnol. 22: 291–298.

WITTENBERG, J. B. 1958. The secretion of inert gas into the swim-bladder of fish. J. Gen. Physiol. 41: 783–804.

Local-Scale versus Large-Scale Factors Affecting Recruitment

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Recent analyses of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks in the northwest Atlantic have indicated that large-scale factors (several hundred to thousands of kilometres) dominate over effects operating on more local scales (10 to a few hundred kilometres) in determining recruitment patterns for these stocks. Adding additional years and additional stocks to the data set, the recruitment time series for cod and haddock have been reexamined. A first differencing technique was used to remove trends in the data. The results indicate that while large-scale effects probably do affect recruitment, the dominant factors are operating on more local scales and correlations are strongest for neighboring stocks.

Les résultats de récentes analyses des stocks de morue de l'Atlantique (*Gadus morhua*) et d'aiglefin (*Melanogrammus aeglefinus*) peuplant les eaux de l'Atlantique nord-ouest révèlent que des facteurs à grande échelle (de plusieurs centaines à plusieurs milliers de kilomètres) affaiblissent les incidences de facteurs locaux (de 10 à quelques centaines de kilomètres) dans la détermination des régimes de recrutement. On a réévalué la série temporelle de données sur le recrutement de la morue et de l'aiglefin en y ajoutant des données recueillies pendant d'autres années et des données sur d'autres stocks. La méthode des différences premières a permis d'éliminer les tendances dans les données. Les résultats révèlent que même si les incidences à grande échelle agissent sur le recrutement, les facteurs dominants agissent sur une échelle plus locale et les corrélations sont plus marquées entre des stocks voisins.

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There has been a great deal of interest in the effects of large-scale factors, such as climate, on fisheries (Cushing 1982; Mysak 1986; Shepherd et al. 1984). Recent work on patterns of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) recruitment in the northwest Atlantic (Koslow 1984; Koslow et al. 1987) has stressed the dominance of large-scale physical and biological forcing over more local biological and physical processes. Large scale refers to processes occurring over several hundred to thousands of kilometres, while local-scale mechanisms operate over 10 to a few hundred kilometres.

An examination of the cod and haddock recruitment data for the northwest Atlantic (see fig. 2 in Koslow 1984, fig. 9 in Koslow et al. 1987, and fig. 1 and discussion in Thompson and Page 1989) shows that there are trends in recruitment for many of the stocks, i.e. the data are not stationary. Stationarity is an underlying assumption of the analysis of time series data (Bartlett 1946; Box and Jenkins 1976). It has been shown (Dickey et al. 1986; Granger and Newbold 1977) that two nonstationary time series often show spurious correlations. The consequences of differencing a stationary series are much less severe, resulting in unbiased and consistent estimates although of reduced power (Dickey et al. 1986). Previous investigators of large-scale synchrony in cod and haddock recruitment in the northwest Atlantic (Koslow 1984; Koslow et al. 1987) did not remove any trends in the data. Rather, the significance levels were adjusted for the autocorrelations in the data using the method of Garrett and Petrie (1981), which is based on that of Bayley and Hammersley (1946). However, this method is applicable only to stationary autocorrelated series (Bayley and Hammersley 1946; Garrett and Petrie 1981). Recently, Thompson and Page (1989) reanalysed the same time series of cod and haddock data as Koslow (1984) and Koslow et al. (1987) using the first differenced time series as suggested by Cohen et al. (1986).

We have reexamined the northwest Atlantic cod and haddock recruitment data, including additional years of recruitment data not used in the other analyses and some stocks for which data were not previously available, using the technique of first differencing to obtain time series that are stationary. Our results suggest that local-scale processes, rather than large-scale physical forcing, dominate the recruitment patterns of cod and haddock in the northwest Atlantic.

Data and Methods

The recruitment of cod stocks from Labrador to Georges Bank and haddock stocks from Browns Bank, the Scotian Shelf, the Gulf of Maine, and Georges Bank is considered. The stock designations correspond to those of the Northwest Atlantic Fisheries Organization (NAFO). These are the same stocks considered by Koslow (1984), Koslow et al. (1987), and Thompson and Page (1989), although we have extended the time series to include more recent data. We have also added

TABLE 1. List of cod and haddock stocks in the northwest Atlantic and sources of data for the number of recruits in the NAFO subareas in the northwest Atlantic.

Species	Area	Reference(s)
Cod	1	S. A. Horsted. NAFO SCR Doc. 82/VI/50
Cod	2J3KL	C. A. Bishop and S. Gavaris. NAFO SCR Doc. 82/IX/111 J. Baird and C. A. Bishop. NAFO SCR Doc. 86/47
Cod	3NO	C. A. Bishop and S. Gavaris. NAFO SCR Doc. 82/VI/57 C. A. Bishop and J. Baird. NAFO SCR Doc. 86/35
Cod	3Ps	C. A. Bishop and S. Gavaris. CAFSAC Res. Doc. 81/39 C. A. Bishop and J. Baird. NAFO SCR Doc. 86/36
Cod	4TVn	M. Sinclair and J. S. Maguire. CAFSAC Res. Doc. 81/41 G. Chouinard and D. Clay. CAFSAC Res. Doc. 85/82
Cod	4VsW	J. J. Maguire, G. A. Yound, and A. F. Sinclair. CAFSAC Res. Doc. 82/40 J. Gagne, A. Sinclair, and C. Dale. CAFSAC Res. Doc. 84/78 A. Sinclair and C. Annand. CAFSAC Res. Doc. 84/46
Cod	5Z	F. Serchuk and S. Wigley. 1986. Woods Hole Lab. Ref. Doc. 86-12
Cod	4X	S. Campana and J. Simon. CAFSAC Res. Doc. 87/30
Haddock	4VW	D. E. Waldron. CAFSAC Res. Doc. 80/61 R. Mahon, P. Simpson, and D. Waldron. CAFSAC Res. Doc. 85/47
Haddock	4X	R. O'Boyle. CAFSAC Res. Doc. 81/24 R. O'Boyle and R. Gregory. CAFSAC Res. Doc. 85/109
Haddock	5Ze	S. Clark et al. J. Northest Atl. Fish. Sci. 3: 127 K. Waiwood and J. Neilson. CAFSAC Res. Doc. 85/95
Haddock	5Y	Overholtz et al. 1983. Woods Hole Lab. Ref. Doc. 83-23

cod from Browns Bank (4X) and Georges Bank (5Z) and haddock from the Gulf of Maine (5Y). The source documents for the data used are listed in Table 1. Recruitment is assumed to be represented by the number of 1-yr-olds in a virtual population analysis (VPA) for all stocks except Gulf of Maine haddock for which there is no VPA. The number of 1-yr-olds in the Northeast Fisheries Centre spring bottom trawl survey was used as an index of recruitment for 5Y haddock. To obtain stationary time series, the technique of first differencing was used. First differencing results in the creation of a new series by taking the difference between successive points. The stationarity of the resulting series was confirmed by an examination of the autocorrelations (Box and Jenkins 1976). Before differencing, a log transformation was used to stabilize the variance and because recruitment data are usually log normal (Hennemuth et al. 1980).

The correlations (Table 2) were done using the longest common time period for each pair of stocks. In all cases the last year used was 1980 to minimize any uncertainties in the VPA results. The series are from 14 to 34 yr long with 13–33 points in the differenced series. The degrees of freedom were not corrected for autocorrelation which should lead to a maximum number of statistically significant correlations, i.e. favoring large-scale effects.

Results

There are only nine statistically significant correlations out of 55 pairings for the differenced time series (Table 2). For the cod stocks there are five significant correlations (coefficients are in parentheses): 3NO and 3Ps (0.63), 3Ps and 4TVn (0.55), 4TVn and 4VsW (0.52), 4VsW and 5Z (0.54), and 4X and 5Z (0.49). Among these the 3NO, 3PS pair was significant at the 95% level. The only significant correlation among haddock stocks is between the 4X and 5Z stocks (0.72), significant at the 99% level, and the others were significant at the 99.9% confidence level. There are three significant correlations between cod and haddock stocks, cod 3NO and haddock 5Z (0.53), cod 3PS and haddock 5Y (0.45), and cod and haddock in 5Z (−0.62). The first two are significant at the 95% level and the last (the only significant negative correlation) at the 99% level.

The number of significant correlations is substantially reduced from previous analyses. Using the original undifferenced (nonstationary) recruitment series, Koslow et al. (1987) found 16 significant correlations out of 36 stock pairs. This analysis of the same series results in only six statistically significant correlations out of 36, but all of the correlations (15) between cod stocks considered by Koslow (1984) and Koslow et al. (1987) are still positive. However, the cod–haddock interactions are no longer all positive (Koslow 1984; Koslow et al. 1987); instead there are equal numbers of negative and positive correlations.

Our results, including the additional stocks, are indicative of regional interactions and suggest that there are two groupings in the range of cod stocks. Stocks 2J3KL, 3NO, 3Ps, 4TVn, and 4VsW are all positively correlated with each other. The more southerly cod stocks on Georges Bank (5Z) and Browns Bank (4X) are significantly correlated with each other and show an equal number of positive and negative correlations with the other cod stocks. The more southerly cod stocks (4TVn, 4VsW, 4X, and 5Z) are, with one exception (4VW), negatively correlated with all haddock stocks outside the Gulf of Maine. The northern cod stocks (1, 2J3KL, 3NO, and 3Ps) are all positively correlated with the haddock stocks except the Gulf of Maine stock (5Y).

Discussion

Our results for cod are similar for the stocks considered by previous investigations of large-scale synchrony in the northwest Atlantic (Table 2); although the number of significant interactions is reduced, all of the cod–cod interactions show positive correlations. The case for synchrony between cod and haddock is much weaker, with only one significant positive correlation; the remainder consists of six negative, one zero, and 10 positive correlations.

The additional stocks added to the analysis indicate that there are regional groupings in recruitment patterns. For example, there are an equal number of positive and negative correlations between the original cod stocks (cod 1 to cod 4VsW) and the Georges Bank (5Z) and Browns Bank (4X) stocks.

The apparent grouping of the cod stocks into two regions suggests the importance of relatively local factors, presumably the local physical oceanographic regimes. The northern group

TABLE 2. Correlation coefficients for cod and haddock stocks in the northwest Atlantic. *Significant at 95% confidence level; **significant at 99%; ***significant at 99.9%. The original 36 pairs of correlations from Koslow (1984) are set off by boxes.

	Cod							Haddock			
	2J3KL	3NO	3PS	4TVn	4VsW	4X[a]	5Z[a]	4VW	4X	5Z	5Y[a]
Cod											
1	0.59	0.22	0.59	0.47	0.26	0.10	0.17	−0.17	0.40	0.26	0.12
2J3KL		0.43	0.43	0.44	0.11	−0.08	−0.24	0.30	0.20	0.19	0.12
3NO			0.63**	0.44	0.28	−0.31	−0.21	0.41	0.46	0.53*	0.33
3Ps				0.55*	0.43	−0.15	0.07	0.12	0.23	0.31	0.45*
4TVn					0.52*	−0.08	0.33	0.00	−0.21	−0.17	0.15
4VsW						0.16	0.54*	−0.02	−0.04	−0.19	0.23
4X[a]							0.49*	0.01	−0.12	−0.43	0.28
5Z[a]								−0.18	−0.32	−0.62	0.39
Haddock											
4VW									0.45	0.28	0.28
4X										0.72***	0.34
5Z											−0.06

[a] New data series used in this analysis.

of cod stocks extends from Greenland southward to the area around Newfoundland (areas 1, 2J3KL, 3Ps, and 3NO). This region is directly influenced by the Labrador Current. The southern grouping consists of cod stocks in the Gulf of St. Lawrence and southwestward to Georges Bank (areas 4TVn, 4VsW, 4X, and 5Z). This region is directly influenced by the outflow of the St. Lawrence River.

Seven of the nine significant correlations in the cod and haddock stocks in this data set (Table 2) are between neighboring stocks or stocks in the same area (e.g. cod and haddock in area 5Z). This result further indicates that local-scale effects dominate over larger scale forcing to determine the recruitment fluctuations in northwest Atlantic cod and haddock stocks.

The fact that the dominant effects on recruitment occur on a relatively small scale is consistent with the findings of Hollowed et al. (1987) who showed several groupings of recruitment patterns in fish stocks from the north Pacific. Since recruitment is a multivariate process with different processes operating to varying degrees in different years, it is not surprising that the local scale of events is more important than the large scale.

Our results do not preclude the existence of climatically induced variations in recruitment which are synchronous over large areas. The available time series are probably too short to unequivocally demonstrate large-scale synchrony. With climatic change having a time scale of 10–20 yr, recruitment time series of at least 20–40 yr are needed. The large number of positive correlations between cod stocks (22 of 28) and between haddock stocks (five of six), albeit nonsignificant, does suggest a weak large-scale coherence. However, underlying, coherent large-scale variation may be overshadowed in the correlation analysis by a few large, locally induced variations, particularly when dealing with short time series.

Additionally the period for which there are the most data, 1960–70, was marked by heavy fishing pressure on many stocks. Recruitment of the stocks was probably influenced by the resulting decrease in spawning biomass. Similar to climatic forcing, the increased fishing pressure was a large-scale phenomenon.

In conclusion, a correlation analysis of recruitment time series of cod and haddock in the northwest Atlantic indicates the importance of local-scale effects in determining recruitment. While there may be large-scale synchrony to recruitment variability, the data available preclude a definitive test of that hypothesis. Exacerbating the difficulty of looking at climate-induced variability are local-scale physical processes, density-dependent changes within the stocks, and major changes in fishing effort.

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References

BARTLETT, M. S. 1946. On the theoretical specification and sampling properties of autocorrelated time-series. J. R. Stat. Soc. Ser. B 8: 27–41.

BAYLEY, G. V., AND J. M. HAMMERSLEY. 1946. The effective number of independent observations in an autocorrelated series. J. R. Stat. Soc. Ser. B 8: 184–197.

BOX, G. E. P., AND G. M. JENKINS. 1976. Time series analysis: forecasting and control. Revised edition. Holden-Day, San Francisco, CA. 575 p.

COHEN, E. B., D. G. MOUNTAIN, AND R. N. O'BOYLE. 1986. Absence of large scale coherence in cod and haddock recruitment in the Northwest Atlantic. ICES C.M. 1986/G:89.

CUSHING, D. H. 1982. Climate and fisheries. Academic Press, New York, NY. 373 p.

DICKEY, D. A., W. R. BELL, AND R. B. MILLER. 1986. Unit roots in time series models: tests and implications. Am. Stat. 40: 12–26.

GARRETT, C., AND B. PETRIE. 1981. Dynamical aspects of the flow through the Strait of Belle Isle. J. Phys. Oceanogr. 11: 376–393.

GRANGER, C. W. J., AND P. NEWBOLD. 1977. Forecasting economic time series. Academic Press, New York, NY. 333 p.

HENNEMUTH, R. C., J. E. PALMER, AND B. E. BROWN. 1980. A statistical description of recruitment in eighteen selected fish stocks. J. Northwest. Atl. Fish. Sci. 1: 101–111.

Hollowed, A. B., K. M. Bailey, and W. S. Wooster. 1987. Patterns in recruitment of marine fishes in the northeast Pacific Ocean. Biol. Oceanogr. 5: 99–131.

Koslow, J.A. 1984. Recruitment patterns in Northwest Atlantic fish stocks. Can. J. Fish. Aquat. Sci. 41: 1722–1729.

Koslow, J.A., K. R. Thompson, and W. Silvert. 1987. Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate. Can. J. Fish. Aquat. Sci. 44: 26–39.

Mysak, L. A. 1986. El Niño, interannual variability and fisheries in the northeast Pacific Ocean. Can. J. Fish. Aquat. Sci. 43: 464–497.

Shepherd, J. G., J. G. Pope, and R. D. Cousens. 1984. Variations in fish stocks and hypotheses concerning links with climate. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 185: 255–267.

Thompson, K. R., and F. H. Page. 1989. Detecting synchrony of recruitment using short, autocorrelated time series. Can. J. Fish. Aquat. Sci. 46: 1831–1838.

Precision of Ages Estimated from Five Bony Structures of Arctic Char (*Salvelinus alpinus*) from the Wood River System, Alaska

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Baker, T. T., and L. S. Timmons. 1991. Precision of ages estimated from five bony structures of Arctic char (*Salvelinus alpinus*) from the Wood River System, Alaska. Can. J. Fish. Aquat. Sci. 48: 1007–1014.

Ages of 32 Arctic char (*Salvelinus alpinus*) were estimated from otoliths (surface-examined and broken-and-burned), vertebrae, cleithra, opercula, and subopercula. Six readers examined each structure three times, and the precision of these estimates among structures and readers was compared with means, aging differences, and analyses of variance. Surface-examined otoliths provided the most precise age estimates and were one of the easiest structures to prepare and read. Broken-and-burned otoliths produced less precise estimates for larger, older Arctic char than for smaller, younger Arctic char. Based on lack of precision, cleithra should probably not be used to age Arctic char. Although mean estimates for the five structures were not significantly different, surface-examined otoliths produced older age estimates than break and burn otoliths. We feel that surface-examined otoliths are best for precise estimation of age of Arctic char but recommend that both otolith methods be compared when aging Arctic char older than 8 yr. Scales were also examined from 30 Arctic char but were not included in the analyses after preliminary examination because age was difficult to interpret (circuli were clear on all scales, but only two or three annuli of uncertain significance could be distinguished).

On a estimé l'âge de 32 ombles chevaliers (*Salvelinus alpinus*) à partir des otolithes (examinées en surface et brûlées après avoir été brisées), des vertèbres, des cleithrums, des opercules et des sous-opercules. Six personnes ont examiné chaque structure trois fois et on a comparé la précision de ces mesures d'une structure à l'autre et d'une personne à l'autre en fonction des moyennes, des différences d'âges et des analyses de la variance. Les otolithes examinées en surface ont fourni les estimations de l'âge les plus précises et étaient les structures les plus faciles à préparer et à examiner. Les otolithes brisées et brûlées ont produit des estimations moins précises pour les ombles chevaliers plus gros et plus âgés. Étant donné la faible précision obtenue avec les cleithrums, cette structure de devrait probablement pas être utilisée pour déterminer l'âge de l'omble chevalier. Même si les estimations moyennes de l'âge au moyen des cinq structures n'étaient pas significativement différentes, les otolithes examinées en surface ont donné des valeurs d'âge plus élevées que les otolithes brisées et brûlées. Nous pensons que la méthode d'examen des otolithes en surface est la meilleure méthode pour déterminer précisément l'âge de l'omble chevalier, mais nous recommandons de comparer les deux méthodes faisant appel aux otolithes lorsqu'il s'agit de déterminer l'âge d'ombles chevaliers de plus de 8 ans. On a également examinés les écailles de 30 ombles chevalier, mais les résultats n'ont pas été inclus dans les analyses après étude préliminaire parce que l'âge était difficile à interpréter avec cette méthode (des stries d'accroissement étaient manifestes sur toutes les écailles, mais on ne pouvait distinguer que deux ou trois annulus dont l'interprétation était difficile).

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Three basic approaches are available to estimate the age of fish: (1) an empirical approach based on direct observations such as mark–recapture studies; (2) a statistical approach based on length frequency distributions; and (3) an anatomical approach based on bony structures from fish (see review of Jearld 1983). The resources (time and money) involved in conducting mark–recapture studies are often prohibitive, and length frequency distributions often do not provide accurate age estimates for long-lived fish (Majkowski and Hampton 1983; Everhart and Youngs 1981; McNew and Summerfelt 1978). Therefore, the most common approach is the use of anatomical structures such as scales and otoliths.

For a structure to be useful for age estimation, it must be both accurate and precise. Accuracy, which is beyond the scope of this study, refers to how close estimated age is to the true age of a fish. Precision is the agreement of age estimates among readers and among replicated readings. A structure that gives consistent age estimates by the same reader and by different readers is preferable to one that gives highly variable age estimates. Accurate and precise ages are especially important for northern fish species, such as Arctic char (*Salvelinus alpinus*) and its close relative the Dolly Varden (*S. malma*[2]), that are typically long lived and slow growing. Anatomical structures for char and other fish species have not been validated in accuracy and precision of age estimates (Nordeng 1961; Beamish and McFarlane 1983, 1987; Barber and McFarlane 1987).

Several bony structures, including otoliths (surface-examined, sectioned, and broken-and-burned), scales, opercula, and fin rays, have been used to estimate age of char (Nielsen 1961; Nordeng 1961; Heiser 1966; Reeves 1969; Baker 1987; Barber and McFarlane 1987). Whereas otoliths have been the preferred structure for Arctic char, there is disagreement over which method (surface-examined, sectioned, or broken-and-burned) provides better age estimates (Barber and McFarlane 1987). Scales and fin rays are considered to be of little value in estimating char ages (Nielsen 1961; Nordeng 1961; Heiser 1966; Barber and McFarlane 1987). Nielsen (1961) examined opercula from juvenile Arctic char and determined that features in operculum patterns did not coincide with fish growth. Vertebrae and cleithra have been used to estimate ages of several other species (Casselman 1974; Harrison and Hadley 1979; Clark 1987; Sharp and Bernard 1988), but not char to our knowledge.

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[2] Because Arctic char and Dolly Varden are so closely related and there have been problems with taxonomic identification (especially in Alaska), the term "char" is used hereafter to include both species unless specified otherwise.

Because of lack of age validation and the problems involved with the use of these structures for estimating ages of char and other species, we evaluated the precision of age estimates for Arctic char[3] in the Wood River system, Alaska, from five bony structures: otoliths, vertebrae, cleithra, opercula, and subopercula. We also examined scales but found them unsuitable for aging Arctic char. We also present a modification of Sharp and Bernard's (1988) sampling standard error to compare the precision of age estimates.

Methods

Field and Laboratory Procedures

Arctic char were collected with a beach seine (50 × 3 m with 2.5-cm bar measure mesh) from the Wood River system, in southwest Alaska, in late September 1986. Fork length (millimetres) and sex were recorded at the time of capture. Scales, sagittal otoliths, thoracic vertebrae, cleithra, opercula, and subopercula were removed from 60 char. Scales were removed from an area just below the posterior part of the dorsal fin above the lateral line of each fish. Both sagittal otoliths were collected from each fish. At least three thoracic vertebrae were removed from a location on the spine below the anterior end of the dorsal fin. One entire gill cover (containing one opercula and subopercula) was removed from each fish. At the time of removal, the structures were partially scraped clean with a knife and then stored in coin envelopes. All structures were cleaned months later with a hard-bristle brush, hot water, and detergent. The structures were returned to coin envelopes until they were examined in the summer of 1987.

Scales were mounted between two glass slides and examined under a dissecting microscope at 25–50 × magnification with reflected light against a dark background.

Whole otoliths were examined under a dissecting microscope at 10–25 × magnification with reflected light in a few drops of water against a black background (Fig. 1). Age was determined by counting the hyaline rings (translucent bands) on the surface of the otolith from the nucleus outward. On some of the otoliths, hyaline rings were difficult to distinguish because of opaque deposits of calcium on the lateral surface. Nordeng (1961) found that grinding the lateral surface removed the opaque deposits and greatly improved readability. He removed the opaque deposits by mounting the otoliths in plastic and grinding the lateral surface of the otoliths with a dentist's drill that had a Carborundum stone affixed. Reeves (1969) improved on the process by making it possible to handle more than one otolith at a time during grinding. We ground the lateral surface of the otoliths by hand. An otolith was placed in a few drops of vegetable oil on a Carborundum honing stone. The otolith was ground by placing pressure on the otolith with the end of a finger and rubbing the otolith back and forth across the stone.

Otoliths were soaked in a 1:1 solution of glycerin and water for about 1 wk before examination. Using the procedure described by Christensen (1964), we cut one otolith from each pair through the nucleus with a scalpel and held it in an alcohol flame until the otolith turned brown. Otoliths were examined under a dissecting microscope at 10–25 × magnification with reflected light and with a drop of vegetable oil placed on the broken-and-burned surface of the otolith (Barber and McFarlane 1987). Age was determined by counting the hyaline rings on the broken-and-burned surface of the otolith from the nucleus outward (Fig. 1).

We examined most opercula, subopercula, and cleithra by holding the structure up to a source of transmitted light (Le Cren 1947; Casselman 1974). Some of the structures from smaller fish were examined against a dark background under a dissecting microscope at 6 × magnification with reflected light. Translucent bands appearing on the opercula, subopercula, and cleithra were considered annuli (Fig. 1). For cleithra, age was estimated by counting the bands from the origin out towards the ventral edge of the anterior blade as recommended by Casselman (1974). For opercula and subopercula, age was estimated by counting the first distinct band out from the posterior rim of the fulcrum and proceeding diagonally towards the posterior margin of the bone as recommended by McConnell (1952).

The centra of the thoracic vertebrae were examined under a dissecting microscope at 5 × magnification with reflected light. Age was determined by counting the concentric, calcified ridges on the inner surface of the vertebrae from the core to the outer edge of the centrum (Fig. 1).

Study Design

Thirty-two sets of structures were selected from the 60 sets originally collected, with one Arctic char randomly chosen from each 10-mm length class (230–620 mm). The 32 fish included 15 males and 17 females. Six readers with different degrees of experience (no experience, experience with other species, and experience with Arctic char) were used in the experiment (Table 1). The readers had the greatest amount of experience with surface-examined otoliths, followed by vertebrae. The six readers read each structure three times, except broken-and-burned otoliths, which were only read by five of the readers. A total of 3360 readings were made ((32 fish × 5 structures × 6 readers × 3 replicates) + (32 fish × 1 structure × 5 readers × 3 replicates)).

Each reader examined the same structure from all fish and then proceeded to the next structure until all the structures (except broken-and-burned otoliths) were examined. All six readers completed the process three times. After surface examination of the otoliths was completed, the otoliths were broken-and-burned and again examined three times by only five of the six readers. The sixth reader was unable to make readings on the broken-and-burned otoliths because the otoliths had fragmented during handling by the first five readers. A seventh person assured that the order in which the structures were exam-

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[3] The Arctic char from the Wood River system are a resident, freshwater stock that is considered to be the western form of Arctic char based on meristics and morphometrics (McPhail 1961; McCart 1980).

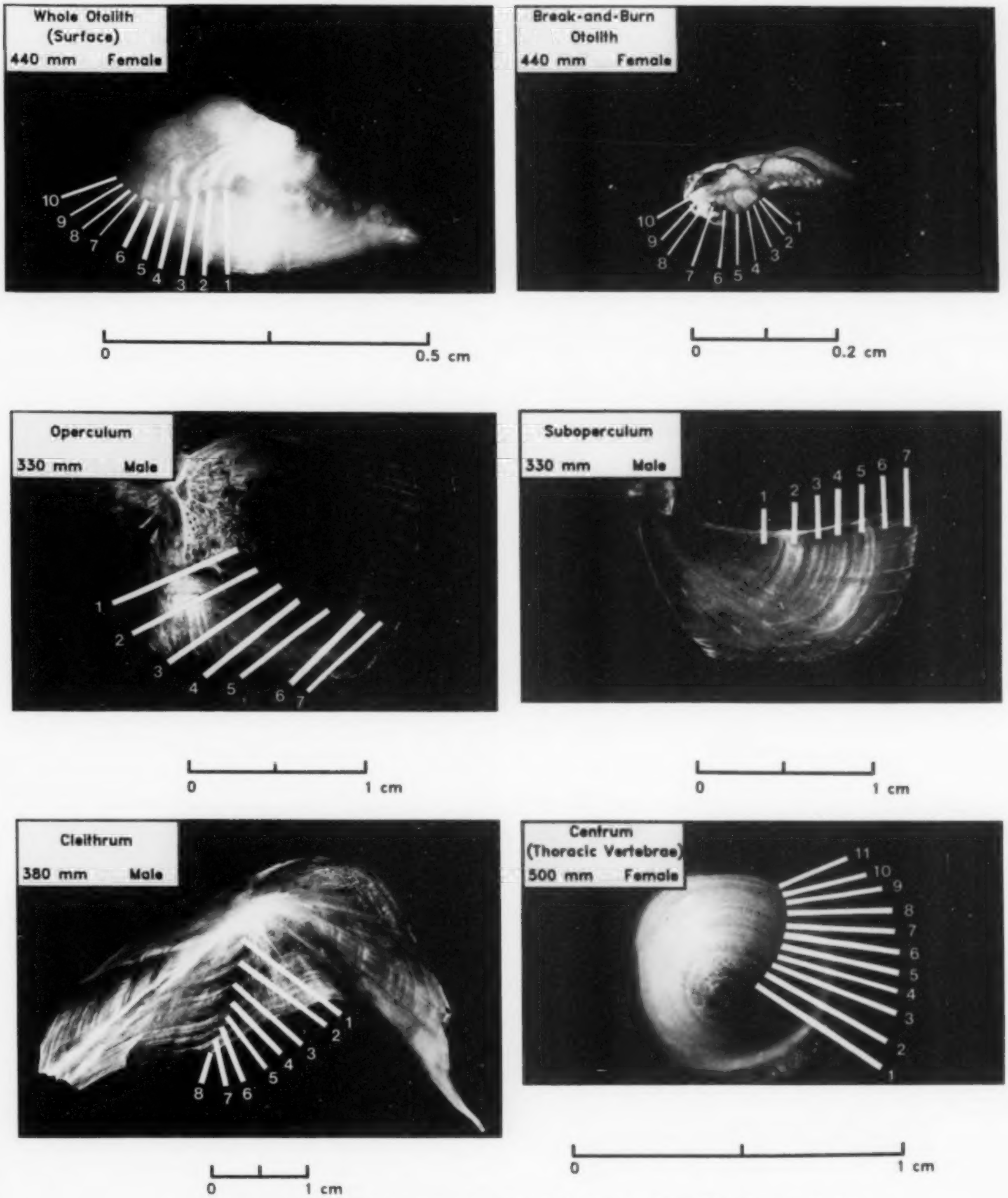
Whole Otolith (Surface)
440 mm Female
10 9 8 7 6 5 4 3 2 1
0 0.5 cm
Break-and-Burn Otolith
440 mm Female
10 9 8 7 6 5 4 3 2 1
0 0.2 cm
Operculum
330 mm Male
1 2 3 4 5 6 7
0 1 cm
Suboperculum
330 mm Male
1 2 3 4 5 6 7
0 1 cm
Cleithrum
380 mm Male
1 2 3 4 5 6 7 8
0 1 cm
Centrum (Thoracic Vertebrae)
500 mm Female
11 10 9 8 7 6 5 4 3 2 1
0 1 cm

FIG. 1. The six structures used to estimate ages of Arctic char from the Wood River System, Alaska.

TABLE 1. Experience of readers with estimating the age of fish from otoliths (surface and break-and-burn), vertebrae, cleithra, opercula, and subopercula. The ordinal values for experience are 0 = no experience, 1 = experience with other species, and 2 = experience with Arctic char.

	Otoliths						
Reader	Surface	Break-and-burn	Vertebrae	Cleithra	Opercula	Subopercula	Mean
1	2	—[a]	1	0	0	0	0.60
2	0	0	0	0	0	0	0.00
3	2	0	1	1	1	0	0.83
4	2	0	2	1	1	0	1.00
5	1	0	0	0	0	0	0.17
6	1	0	1	1	1	0	0.67
Mean	1.33	0.00	0.83	0.50	0.50	0.00	0.54

[a]Reader 1 did not estimate ages using broken-and-burned otoliths.

ined was randomly selected and that the 32 sets of structures were randomly ordered for each reading. Most of the replicate readings were at least 1 d apart. Readers were unaware of the length and sex of the Arctic char and the age assigned the structures in their previous readings or by other readers.

Comparisons of Structures and Readers

Mean age estimates

Mean age estimates for structure–reader combinations, individual structures, and individual readers were compared among structures and readers. The mean age for each structure–reader combination ($\bar{X}_{kl}$) was estimated by summing the age estimates (X_{ijkl}) across fish (f) and replicates (n) and dividing by nf:

$$(1)\quad \bar{X}_{kl} = \frac{\sum_{i}^{n} \sum_{j}^{f} X_{ijkl}}{nf}.$$

Estimated mean age from each structure ($\bar{X}_k$) was $\bar{X}_{kl}$ summed across readers with the sum divided by r, where r was the number of readers. Estimated mean age for each reader ($\bar{X}_l$) was $\bar{X}_{kl}$ summed across structures with the sum divided by s, where s was the number of structures in the experiment.

Aging error

The aging errors (AE) for each structure–fish combination (AE[Structure–Fish]), structure–reader combination (AE[Structure–Reader]), structure (AE[Structure]), and reader (AE[Reader]) were used as indices of the repeatability of age determinations for a comparison of structures and readers. The aging error was determined by first calculating the variance for each structure–reader–fish combination ($V[\bar{X}_{jkl}]$), which was the mean square error summed across n replicates and divided by $(n - 1)$ degrees of freedom:

$$(2)\quad V[\bar{X}_{jkl}] = \frac{\sum_{i}^{n} (X_{ijkl} - \bar{X}_{jkl})^2}{(n - 1)}$$

where $\bar{X}_{jkl}$ was the mean age estimate for the jth fish, kth structure, and lth reader. The variance for each structure–fish combination was $V[\bar{X}_{jkl}]$ summed across readers and divided by r. The variance for each structure was $V[\bar{X}_{jkl}]$ summed over readers and fish, with the sum divided by rf. The variance for each reader was $V[\bar{X}_{jkl}]$ summed over structures and fish, with the sum divided by sf. The aging error for these combinations was the square root of their respective variances.

Analysis of variance

Analysis of variance (ANOVA) was used to compare the mean of age estimates from five structures (surface-examined otoliths, vertebrae, cleithra, opercula, and subopercula) and six readers (Zar 1984). The ANOVA was a balanced design with structures as fixed effects, readers as random effects, and fish as an exogenous source of variation. During this study, a small number of specimens, in addition to the broken-and-burned otoliths, were broken. The balance of the design was maintained by inserting marginal means for missing data into the analysis and appropriately decreasing the degrees of freedom in the hypothesis tests. For example, if one replicate estimate was missing for a structure–reader combination, the average of the other two replicates was used as a replacement. Twenty-eight (0.8%) of the 3360 estimates were missing and replaced with marginal means.

An unbalanced ANOVA was used to compare mean estimates of age from surface-examined and broken-and-burned otoliths (Zar 1984). Otoliths were considered fixed effects and readers were considered random effects; fish were treated as exogenous sources of variation. Broken-and-burned otoliths were excluded from the ANOVA on the other structures because inclusion would have greatly unbalanced the design of that experiment. An unbalanced ANOVA was not used for the entire experiment because of the excessive amounts of computer resources required to process 3810 data.

Results

Comparison of Structures

After examining scales from 30 Arctic char, we eliminated scales from the study because age was difficult to interpret. Although circuli were clearly present on all the scales, only two or three annuli could be distinguished on the scales and these were difficult to interpret.

Estimates of mean age were not as variable among structures as among readers within each structure (Table 2). Otoliths (surface-examined), opercula, subopercula, and cleithra provided the older age estimates, averaging approximately 9.0 yr. The mean age for vertebrae was the lowest at 8.1 yr, with broken-and-burned otoliths intermediate at 8.4 yr. Ranges of mean age estimates among readers were greatest for cleithra and opercula. Subopercula, vertebrae, and surface-examined otoliths were intermediate, with ranges of 3.9, 3.3, and 2.4 yr, respectively. Broken-and-burned otoliths had the smallest range (1.4 yr) among readers. Although broken-and-burned otoliths

TABLE 2. Estimates of mean age in years from the sample of 32 Arctic char for each structure–reader combination, each structure, and each reader.

	Otoliths						Reader	
Reader	Surface	Break-and-burn	Vertebrae	Cleithra	Opercula	Subopercula	Mean	Range
1	10.3	—[a]	9.5	10.2	9.6	9.2	9.7	1.1
2	8.5	8.2	8.6	10.5	8.5	9.6	9.1	2.3
3	7.9	8.3	6.2	6.4	7.1	7.1	7.2	2.1
4	10.0	9.2	7.2	9.7	8.8	9.5	9.1	2.8
5	8.6	8.6	9.5	10.8	11.2	11.0	9.9	2.6
6	8.3	7.8	7.3	8.1	7.4	8.2	7.8	1.0
Structure								
Mean	8.9	8.4	8.1	9.3	8.8	9.1	8.8	1.2
Range	2.4	1.4	3.3	4.4	4.1	3.9	2.8	

[a] Reader 1 did not estimate ages using broken-and-burned otoliths.

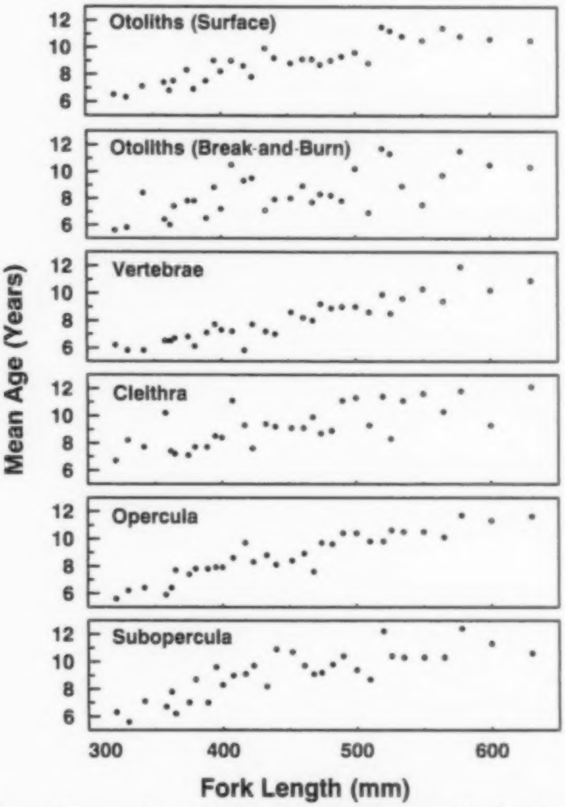

FIG. 2. Mean age for each Arctic char structure–fish combination plotted versus fork length. Each mean is the average of 18 age estimates (6 readers × 3 replicates), except broken-and-burned otoliths which is the average of 15 age estimates (5 readers × 3 replicates).

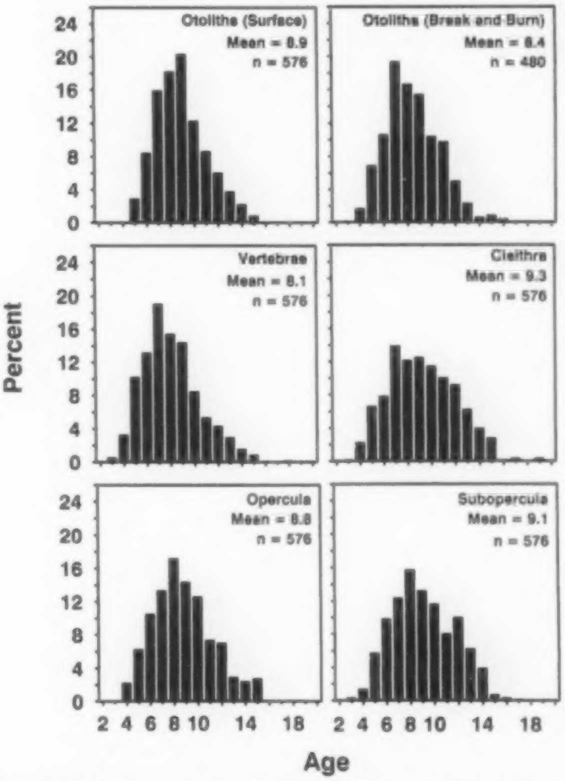

FIG. 3. Distributions of Arctic char age estimates from surface-examined otoliths, broken-and-burned otoliths, vertebrae, cleithra, opercula, and subopercula. Each distribution consists of 576 age estimates (6 readers × 32 fish × 3 replicates), except broken-and-burned otoliths which consists of 480 age estimates (5 readers × 32 fish × 3 replicates).

had the smallest range of means among readers, mean age for broken-and-burned otoliths appeared to be most variable with respect to fish length (Fig. 2). Age estimates for all the other structures increased more regularly with length of fish (Fig. 2).

Distributions of age estimates from all structures were similar (Fig. 3). All age distributions were skewed to younger ages, although the distribution for cleithra was the least skewed. The distribution for vertebrae was almost identical to the distribution of surface-examined otoliths, but was shifted 1 yr younger.

Surface-examined otoliths had the lowest AE for structure of 1.08 yr, followed by broken-and-burned otoliths and vertebrae, opercula, subopercula, and cleithra (1.68 yr) (Table 3). The range of AE for structure among readers was lowest for surface-examined otoliths (0.79 yr), followed by superopercula, vertebrae, opercula, cleithra, and broken-and-burned otoliths (1.25 yr) (Table 3). The AE for structure–fish appeared to increase for broken-and-burned otoliths (Fig. 4). The AE for

TABLE 3. Aging error (AE) in years for estimates of age from the sample of 32 Arctic char for each structure–reader combination, each structure, and each reader.

	Otoliths						Reader	
Reader	Surface	Break-and-burn	Vertebrae	Cleithra	Opercula	Subopercula	SE	Range
1	0.66	—[a]	0.66	1.02	0.98	0.98	0.86	0.36
2	1.45	1.93	1.71	1.91	1.19	1.59	1.63	0.74
3	0.88	1.06	1.30	1.67	1.06	1.50	1.24	0.79
4	0.79	1.25	1.19	1.86	1.27	1.28	1.27	1.07
5	1.44	1.12	1.48	2.22	2.09	1.83	1.69	1.11
6	0.98	0.68	1.02	0.98	0.98	1.32	0.99	0.64
Structure								
SE	1.08	1.27	1.27	1.68	1.32	1.44	1.34	0.60
Range	0.79	1.25	1.05	1.25	1.11	0.85	0.83	

[a]Reader 1 did not estimate ages using broken-and-burned otoliths.

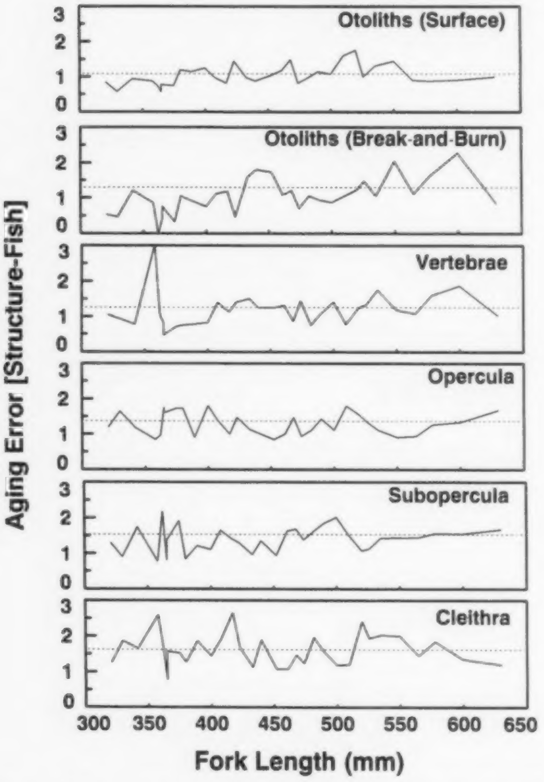

FIG. 4. Aging error (AE), by length, for estimates of age from the sample of 32 Arctic char for each structure–fish combination (AE[Structure–Fish]). The broken line indicates the mean AE[structure] for the 32 Arctic char.

structure-fish for cleithra was larger and more variable than for the other structures (Fig. 4).

Based on the ANOVA, mean ages obtained from five of the structures, surface-examined otoliths, vertebrae, cleithra, opercula, and subopercula, were not significantly different ($F = 2.25$, df $= 4$, $P > 0.0996$) at $\alpha = 0.05$. However, based on Duncan's multiple comparisons test of the least significant differences ($\alpha = 0.05$), the structures were placed in two overlapping groups. Cleithra, subopercula, otoliths (surface-examined), opercula, and vertebrae were placed in the other group. Based on the unbalanced ANOVA, no significant differences were found between surface-examined otoliths and broken-and-burned otoliths ($F = 1.54$, df $= 1$, $P > 0.2826$) at $\alpha = 0.05$.

Comparison of Readers

Mean age estimates for the readers across all structures ranged from 7.2 to 9.9 yr (Table 2). The range of mean ages for the readers across structures was variable. Based on the ANOVA, the age estimates were significantly different ($F = 69.96$, df $= 5$, $P < 0.0001$) between the readers at $\alpha = 0.05$. Based on Duncan's multiple comparisons of the least significant differences ($\alpha = 0.05$), the readers were split into five categories, with readers two and four grouped in the same category and readers one, three, five, and six grouped in separate categories. Based on the unbalanced ANOVA comparing mean ages for the six readers for both techniques for estimating ages from otoliths, a significant difference ($F = 25.61$, df $= 5$, $P < 0.0001$) was found between readers at $\alpha = 0.05$. Multiple comparisons of least significant differences ($\alpha = 0.05$) placed readers one and four into separate groups, while the other readers were split into two overlapping groups, one containing readers two and five and the other containing readers two, three, and six.

Even though age estimates by readers were varied within structures, no correlation was found between overall reader experience (Table 1) and precision (Table 3) for all the structures except surface-examined otoliths. Readers more experienced in reading surface-examined otoliths (readers one, three, and four) had lower AE for structure–reader than readers with no experience.

Discussion

A modified method, or index, was used in this study to measure the precision of age estimates. The index, called the *aging error* (AE), was modified from Sharp and Bernard's (1988) sampling standard error and was calculated for each structure-fish combination (AE[Structure–Fish]), structure–reader combination (AE[Structure–Reader]), individual structure (AE[Structure]), and individual reader (AE[Reader]). Each AE was used to measure and compare the precision of age estimates in relation to fish, structure, reader, or a combination of these components. Those structures that have the largest "differences" are the least desirable for use in estimating the age of a fish. The AE is a measure of those "differences." The AE[Structure–Fish] is identical to the sampling standard error proposed by Sharp and Bernard (1988) and is analogous to the average percent error (APE) proposed by Beamish and Fournier (1981) and the coefficient of variation (CV) proposed by Chang (1982). However, the AE for all the combinations differs from the APE and the CV because they are not scaled to the age of the fish. Also, the AE[Structure–Fish] differs from the APE and the CV because it is oriented to the fish in addition to the reader.

The ambiguity often encountered in determining ages of fish from anatomical structures can be seen in Fig. 1. Although some structures may be better than others, all structures presented some amount of uncertainty when they were used for age determination, even under the best of circumstances. The surface and break-and-burn otoliths were from the same fish. In Fig. 1, age was estimated to be 10 yr from surface and break-and-burn otoliths. However, the age of the fish could be as high as 15 or 16 yr if all the narrow rings were counted on the outer edge of the whole otolith. In this case, we think the number of rings was not representative of the age of the fish. After age 5 or 6, Arctic char begin to spawn and we believe that some of the rings on the outer edge of the surface-examined otolith may be spawning checks. However, until a study of the accuracy of the structures can be conducted, we will not know either way. It is important to note that the surface-examined otolith in Fig. 1 represents the clearest picture of the otoliths. The narrow rings near the edge of that surface-examined otolith were not observed in the majority of otoliths used in this study. The operculum and suboperculum shown in Fig. 1 were from the same fish, but the ages assigned to this ranged from 4 to 14 yr for the operculum and from 3 to 8 yr for the suboperculum. In Fig. 1, seven annuli are indicated on the operculum, but several more markings between the third and sixth annuli could also be considered annuli. In addition, the first and last annuli were questionable. Markings on the suboperculum were similarly ambiguous. Particularly uncertain were the markings between the third and the seventh annuli, and the innermost annulus was also dubious. Vertebrae and cleithra presented difficulties similar to the other structures. The markings near the core of the vertebrae were the most difficult to interpret (Fig. 1). For cleithra, the interpretation of annuli was difficult along the entire structure, with interpretation near the origin the most difficult (Fig. 1).

Surface-examined otoliths appeared to be the preferred structure to estimate age of Arctic char because they provided the most precise age estimates and were one of the easiest structures to prepare and read. Although mean ages by structure were not significantly different, age estimates from vertebrae were approximately 1 yr younger than ages from the other structures. The difference of 1 yr for vertebrae may be attributed to the absence of a first-year annulus or the difficulty of detecting the first annulus. These problems have been associated with scales (Alvord 1954; Linfield 1974) and opercula (Le Cren 1947; McConnell 1952; Neuhold 1956; Frost and Kipling 1959) in other species.

Barber and McFarlane (1987) estimated the ages of fish from three stocks of Arctic char in North America from surface-examined and break-and-burn otoliths. The surface-examined and break-and-burn otoliths produced similar estimates for Arctic char up to 8 yr of age. However, for Arctic char 8 yr and older, break-and-burn otoliths produced older age estimates than surface-examined otoliths. Because of this, they recommended that Arctic char older than 8 yr be aged by break-and-burn otoliths and went on to suggest that ages determined from surface examination of otoliths underestimate the true ages of Arctic char over age 8. Contrary to Barber and McFarlane (1987), age estimates from surface-examined otoliths in this study were older and more precise than ages estimated from break-and-burn otoliths (Fig. 2). The different results we obtained may be related to our technique for preparing otolith surfaces (Nordeng 1961; Reeves 1969; Baker 1987). We ground the lateral surface of the otolith near the focus, a procedure which removed the opaque deposits near the focus and exposed additional annuli. The older mean ages obtained from surface examination in this study may also be a reflection of reader experience because the readers in this study had no experience with break-and-burn otoliths, while five of the six readers had previous experience with surface-examined otoliths. Even with the differences in reader experience, we feel that surface-examined otoliths are best for precise estimation of age of Arctic char. Otoliths are easily prepared and examined with the surface-examination method used in this study. Also, char otoliths are small and difficult to break and burn. For these reasons, we recommend that surface-examined otoliths be used to estimate the age of Arctic char in the future.

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References

ALVORD, W. 1954. Validity of age determination from scales of brown trout, rainbow trout, and brook trout. Trans. Am. Fish. Soc. 80: 91–103.

BAKER, T. T. 1987. Age and growth of Arctic char in the Wood River system, Alaska. M.S. thesis, University of Alaska Southeast, Juneau, AK. 94 p.

BARBER, W. E., AND G. A. MCFARLANE. 1987. Evaluation of three techniques to age Arctic char from Alaskan and Canadian waters. Trans. Am. Fish. Soc. 116: 874–881.

BEAMISH, R. J., AND D. A. FOURNIER. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38: 982–983.

BEAMISH, R. J., AND G. A. MCFARLANE. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735–743.

1987. Current trends in age determination methodology, p. 15–42. *In* R. C. Summerfelt and G. E. Hall [ed.] Age and growth of fish. Iowa State University Press, Ames, IA.

CASSELMAN, J. M. 1974. Analysis of hard tissue of pike *Esox lucius* L. with special reference to age and growth, p. 13–27. *In* T. B. Bagenal [ed.] Proceedings of an International Symposium on the Ageing of Fish. Gresham Press, Old Woking, England.

CHANG, W. Y. 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39: 1208–1210.

CHRISTENSEN, J. M. 1964. Burning of otoliths, a technique for age determination of soles and other fish. J. Cons. 29: 73–81.

CLARK, R. A. 1987. Sources of variability in three aging structures for Yukon River fall chum salmon (*Oncorhynchus keta* Walbaum) escapement samples, p. 111–121. *In* Proceedings of the 1987 Northeast Pacific Pink and Chum Salmon Workshop. Alaska Department of Fish and Game, Juneau, AK. 223 p.

EVERHART, W. H., AND W. D. YOUNGS. 1981. Principles of fisheries science. Cornell University Press, Ithaca, NY. 349 p.

FROST, W. E., AND C. KIPLING. 1959. The determination of the age and growth of pike (*Esox lucius* L.) from scales and opercular bones. J. Cons. Int. Explor. Mer 24: 314–341.

HARRISON, E. J., AND W. F. HADLEY. 1979. A comparison of the use of cleithra to the use of scales for age and growth studies. Trans. Am. Fish. Soc. 108: 452–456.

HEISER, D. W. 1966. Age and growth of anadromous Dolly Varden char in Eva Creek, Baranof Island, Southeastern Alaska. Alaska Dep. Fish Game Res. Rep. No. 5.

JEARLD, A. JR. 1983. Age determination, p. 301–324. *In* L. A. Nielsen and D. L. Johnson [ed.] Fishery techniques. American Fisheries Society, Bethesda, MD. 468 p.

LE CREN, E. D. 1947. The determination of the age and growth of the perch (*Perca fluviatilis*) from the opercular bone. J. Anim. Ecol. 16: 188–204.

LINFIELD, R. S. J. 1974. The errors likely in aging roach *Rutilus* (L.) with special reference to stunted populations, p. 167–172. *In* T. B. Bagenal [ed.] Proceedings of an International Symposium on the Ageing of Fish. Gresham Press, Old Woking, England.

MAJKOWSKI, J., AND J. HAMPTON. 1983. The effect of parameter uncertainties in an age–length relationship upon estimating the age composition of catches. Can. J. Fish. Aquat. Sci. 40: 272–280.

MCCONNELL, W. J. 1952. The opercular bone as an indicator of age and growth in carp, *Cyprinus carpio* Linnaeus. Trans. Am. Fish. Soc. 81: 138–149.

MCCART, P. J. 1980. A review of the systematics and ecology of Arctic char, *Salvelinus alpinus*, in the western Arctic. Can. Tech. Rep. Fish. Aquat. Sci. 935: 89 p.

MCNEW, R. W., AND R. C. SUMMERFELT. 1978. Evaluation of a maximum-likelihood estimator for analysis of length–frequency distributions. Trans. Am. Fish. Soc. 107: 730–736.

MCPHAIL, J. D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. J. Fish. Res. Board Can. 18: 793–814.

NEUHOLD, J. M. 1956. Age and growth of the Utah chub, *Gila atraria* (Girard), in Panguitch Lake and Navajo Lake, Utah from scales and opercular bones. Trans. Am. Fish. Soc. 85: 217–233.

NIELSEN, J. 1961. Contributions to the biology of the Salmonidae in Greenland. IV. Age and growth of the freshwater stage of anadromous char, *Salvelinus alpinus* (L.). Medd. Gronl. 159: 49–75.

NORDENG, H. 1961. On the biology of char (*Salmo alpinus* L.) in Salangen, north Norway. 1. Age and spawning frequency determined from scales and otoliths. Nytt Mag. Zool. (Oslo) 10: 67–123.

REEVES, J. E. 1969. A comparative analysis of Arctic char populations in sockeye salmon lakes of the Nushagak District, Alaska. Ph.D. thesis, University of Washington, Seattle, WA.

SHARP, D., AND D. R. BERNARD. 1988. Precision of estimated ages of lake trout from five structures. N. Am. J. Fish. Manage. 8: 367–372.

ZAR, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ. 620 p.

Variation within and between Rivers in Adult Size and Sea Age at Maturity of Anadromous Brown Trout, *Salmo trutta*

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I compared adult size and sea age at sexual maturity among nine populations of anadromous brown trout, *Salmo trutta*, in two Norwegian rivers to determine the extent of inter- and intrariverine variations. Variation in the mean length of spawners and in the mean sea age at sexual maturity were mainly dependent on the variations found within rather than between rivers. Mean lengths and mean age at maturity of males increased significantly with increasing altitude of the spawning area and with migration distance in freshwater. In females, positive significant correlations were found with mean lengths and altitude of the spawning area and with mean sea age at maturity and both spawning site altitude and migration distance. Mean lengths and ages of males and females were not significantly correlated with the rate of water discharge in the streams during spawning. The size of gravel substrate for spawning was of minor importance in explaining interpopulation variation in mean female size. The increase noted in mean length and in mean sea age at maturity of both males and females is probably an adaptation to greater energy expenditure to reach the uppermost natal spawning areas.

On a comparé la taille à l'âge adulte le temps passé en mer à la maturité sexuelle de truites brunes, *Salmo trutta*, anadromes appartenant à neuf populations vivant dans deux rivières de la Norvège afin d'étudier les variations dans une même rivière et entre rivières. On a constaté que la variation de la longueur moyenne des reproducteurs et du temps moyen passé en mer à la maturité sexuelle dépendent essentiellement des variations dans une même rivière plutôt qu'entre rivières. La longueur moyenne et le temps moyen à la maturité chez les mâles augmentaient dans une mesure significative en fonction de l'altitude du lieu de fraye et de la distance de migration en eau douce. Chez les femelles, on a constaté des corrélations positives significatives entre la longueur moyenne et l'altitude du lieu de fraye de même qu'entre le temps moyen en mer à la maturité et l'altitude du lieu de fraye ainsi que la distance de migration. Chez les mâles et les femelles, il n'est pas apparu de corrélation significative entre la longueur moyenne et le temps passé en mer et le débit des cours d'eau durant la fraye. La granulométrie du gravier de fraye s'est révélé n'avoir qu'une influence mineure sur la variation de la taille moyenne des femelles d'une population à l'autre. L'augmentation de la longueur moyenne et du temps moyen passé en mer à la maturité constatée tant chez les mâles que chez les femelles est probablement une adaptation découlant de ce qu'il faut une dépense énergétique est plus importante pour atteindre les eaux de fraye d'origine les plus élevées.

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Interriverine variation in size of Atlantic salmon, *Salmo salar*, has been related to environmental factors such as the migratory distance in freshwater to the natal spawning areas and the rate of water discharge (Schaffer and Elson 1975; Thorpe and Mitchell 1981; Scarnecchia 1983). As migration distance and river discharge determine the energy used to reach the spawning ground, Schaffer and Elson (1975) suggested that long, large rivers would favor selection for larger sized fish with greater energy reserves. One consequence of this could be that within rivers the larger fish may spawn in the upper part and the smaller fish further downstream. Glebe and Leggett (1981) demonstrated in American shad, *Alosa sapidissima*, a higher energy cost of migration in smaller fish. However, in several anadromous fish species (American shad, Atlantic salmon, and coho salmon, *Oncorhynchus kisutch*) and the results are contrary to Schaffer and Elson's (1975) prediction (Carscadden and Leggett 1975; Scarnecchia 1983; Fleming and Gross 1989). In a recent study of 34 populations of anadromous brown trout, *Salmo trutta*, L'Abée-Lund et al. (1989) found no significant correlation of adult fish length with river length and water discharge. However, it was pointed out that such relationships may nevertheless exist because the precise spawning areas for many of the populations were unknown. The homing behavior of brown trout for its natal spawning area (Stuart 1957; Hindar et al. 1991) is of great importance to maintain any intrariverine variation in adult size and sea age at sexual maturity.

Salmonids are selective in choosing the substrate for spawning by avoiding areas with silt and clay. Reiser and Bjornn (1979) stated that in salmonids the suitability of a particular size gravel substrate depended mostly on fish size. However, Ottaway et al. (1981) demonstrated that the grain size of brown trout redds was not related to the fish length.

In this paper I compared the variation in sea age at sexual maturity and size of adult anadromous brown trout from nine populations, whose spawning areas were known, in two rivers. The null hypotheses was that (1) the sea age at sexual maturity and adult size were independent of increasing migration distance, spawning site altitude, and mean water discharge during the spawning season in the spawning area and (2) the length of females was not related to the grain sizes within redds.

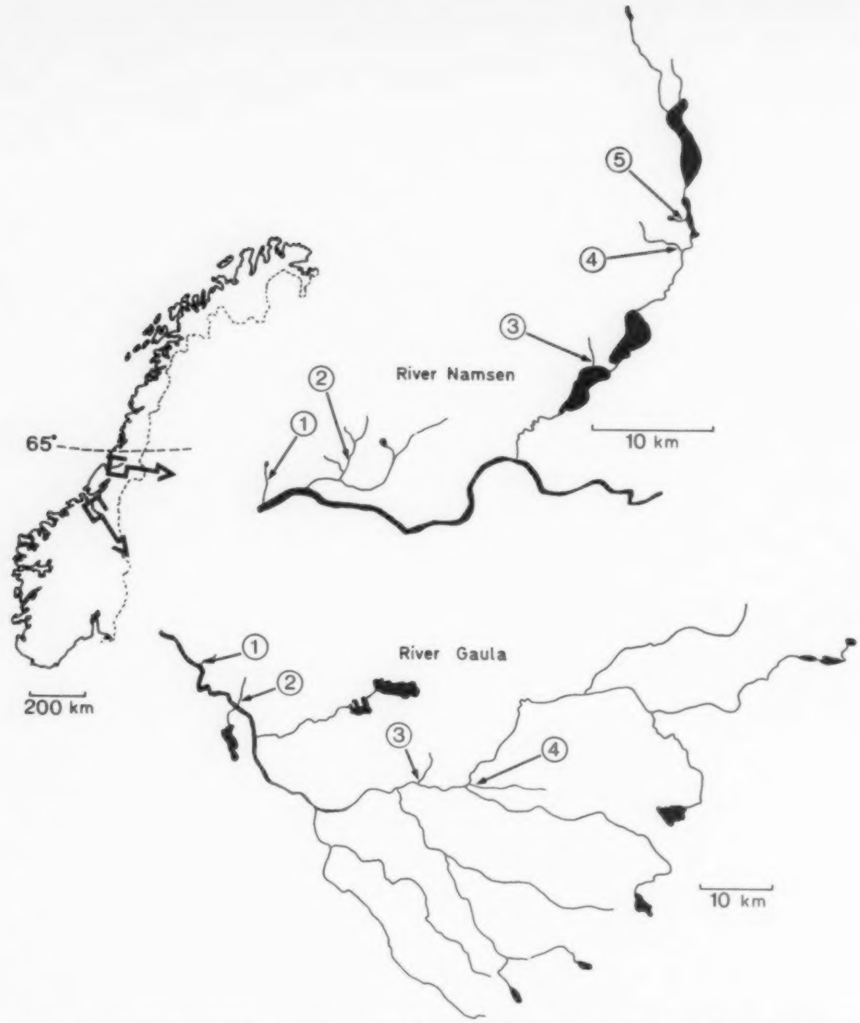

FIG. 1. Locations of the nine tributary streams of the two Norwegian rivers in which the populations of anadromous brown trout were studied. Tributary names are shown in Table 1.

Materials and Methods

Sampling

Anadromous brown trout were investigated in nine tributaries of two Norwegian rivers, the River Gaula and the River Namsen, situated at 63°20′N and 64°28′N, respectively (Fig. 1). The distance between tributaries along the main river varied from approximately 4 to 44 km (Table 1). Fish were sampled during the spawning season, i.e. September–October, by electrofishing. Fish were caught while spawning, as all males had running milt, and most females had running roe or were spent. The remaining females were almost ready to ovulate. Local homing in brown trout has been found when it was looked for (e.g. Stuart 1957; Halvorsen and Stabell 1990; Hindar et al. 1991). Thus, I define the individuals spawning within each stream to form a population (Mayr 1970).

Traits

Each fish was measured (total length) to the nearest millimetre and sex and degree of sexual maturity were recorded (Dahl 1917). Ages were determined from scales (Jonsson 1985). Determination of age at sexual maturity was based on scale readings (Dahl 1910). Comparisons of mean age and size at smolting revealed that the two uppermost populations in both rivers smoltified at a significantly (Scheffé procedure on means, $P < 0.05$) greater age and size than those in the lowermost populations (Table 2). Within populations, no significant differences ($P > 0.05$) were found in smolt age and size between sexes. Thus, to reduce the effect of freshwater history, age at sexual maturity was expressed as the number of summers spent at sea before reaching sexual maturity.

Environmental Parameters

In each of the four tributaries of the River Gaula, two to seven samples of gravel substrate (3227 – 11 687 g) were analysed. If possible, redds were sampled. Samples were dry screened through three sieves (2, 6.3 and 20.0 mm), and the structure of spawning substrate within tributaries are expressed as mean frequency of different grain sizes.

TABLE 1. Tributary streams sampled, migration distance (distance from the sea to the tributary), spawning site altitude, mean rate of water discharge (minimum–maximum) in the tributaries during spawning season, and sampling period for nine populations of anadromous brown trout from two Norwegian rivers.

Location	Migration distance (km)	Altitude (m)	Water discharge (m^3/s)	Sampling period
River Gaula				
1. Loddbekken	9.9	20	0.16 (0.03–0.38)	1986–88
2. Bortna	22.1	40	0.16 (0.02–0.38)	1986–88
3. Malma	59.2	135	0.27 (0.03–0.90)	1989
4. Herjåa	67.6	170	0.92 (0.11–3.13)	1988–89
River Namsen				
1. Mølleelven	0.6	10	0.18 (0.03–0.42)	1989
2. Norddalselven	6.9	10	1.24 (0.17–2.96)	1989
3. Vikabekken	51.0	10	0.27 (0.04–0.61)	1986
4. Almåselven	70.0	40	0.49 (0.07–1.12)	1986–89
5. Skeitjørnbekken	73.9	65	0.15 (0.02–0.33)	1986

TABLE 2. Smolt age and size (arithmetic means ± 95% C.L. and ranges) for nine anadromous brown trout populations from two Norwegian rivers.

	Age (yr)			Size (cm)		
Location	*n*	Mean	Range	*n*	Mean	Range
River Gaula						
1. Loddbekken	64	3.0 ± 0.18	2–5	64	15.1 ± 0.92	8–24
2. Bortna	56	2.8 ± 0.14	2–4	56	13.8 ± 0.97	8–26
3. Malma	15	3.6 ± 0.41	2–5	15	17.5 ± 1.88	12–24
4. Herjåa	28	3.7 ± 0.26	3–5	30	16.2 ± 1.04	12–26
River Namsen						
1. Mølleelven	33	2.6 ± 0.22	2–4	34	12.3 ± 1.08	8–17
2. Norddalselven	32	2.8 ± 0.23	2–4	32	13.4 ± 1.05	7–19
3. Vikabekken	22	3.5 ± 0.36	2–5	22	17.8 ± 1.55	12–22
4. Almåselven	59	3.8 ± 0.21	2–6	61	20.0 ± 0.99	7–30
5. Skeitjørnbekken	23	3.6 ± 0.45	2–6	23	18.7 ± 2.18	11–28

TABLE 3. Lengths of adult fish and sea ages at sexual maturity (arithmetic means ± 95% C.L. and ranges) for nine anadromous brown trout populations from two Norwegian rivers. Asterisks indicate significant ($P < 0.05$) differences in length and age between sexes within populations.

	Males			Females		
Location	*n*	Length (cm)	Age (summers at sea)	*n*	Length (cm)	Age (summers at sea)
River Gaula						
1. Loddbekken	48	36.7 ± 2.14 (25–56)	2.0 ± 0.11 (1–3)	16	43.0 ± 4.10 (31–59)*	2.4 ± 0.33 (1–3)*
2. Bortna	42	36.6 ± 2.12 (24–50)	2.0 ± 0.14 (1–3)	14	42.7 ± 3.70 (32–53)*	2.6 ± 0.43 (2–4)*
3. Malma	8	47.6 ± 7.30 (29–56)	2.6 ± 0.62 (2–4)	7	53.2 ± 6.36 (43–63)	2.7 ± 0.45 (2–3)
4. Herjåa	12	52.7 ± 5.58 (41–69)	2.9 ± 0.50 (2–4)	18	53.3 ± 3.12 (41–61)	3.3 ± 0.29 (2–4)
River Namsen						
1. Mølleelven	24	33.5 ± 2.21 (24–45)	2.1 ± 0.19 (1–3)	12	36.2 ± 3.13 (31–46)	2.3 ± 0.29 (2–3)
2. Norddalselven	22	37.7 ± 2.97 (25–48)	2.5 ± 0.31 (2–4)	13	42.3 ± 2.75 (36–52)*	2.5 ± 0.31 (2–3)
3. Vikabekken	9	35.5 ± 3.81 (27–41)	2.1 ± 0.46 (1–3)	13	38.8 ± 4.82 (24–51)	2.0 ± 0.53 (1–4)
4. Almåselven	42	41.3 ± 1.70 (28–59)	2.8 ± 0.34 (1–4)	25	41.9 ± 2.00 (35–57)	2.9 ± 0.32 (2–4)
5. Skeitjørnbekken	18	43.9 ± 2.18 (34–51)	2.5 ± 0.39 (2–4)	5	42.8 ± 8.29 (36–50)	2.6 ± 0.68 (2–3)

The climatic differences between the two rivers (i.e. water temperature, precipitation, and photoperiod) are probably small due to their relatively close proximity. The rate of water discharge (cubic metres per second) in each spawning area during the spawning season was estimated. This was done by estimating the drainage area of each tributary from topographic maps and relating this to the total drainage area of the river and the water discharge measured by the Norwegian Water and Electricity Board, Hydrological Department, in the river's catchment basin during the spawning season. Mean water discharge during spawning season varied among tributaries from 0.15 to 1.24 m^3/s (Table 1). Migration distance (kilometres) is the distance that the brown trout of each population have to migrate in freshwater from the sea to the natal spawning trib-

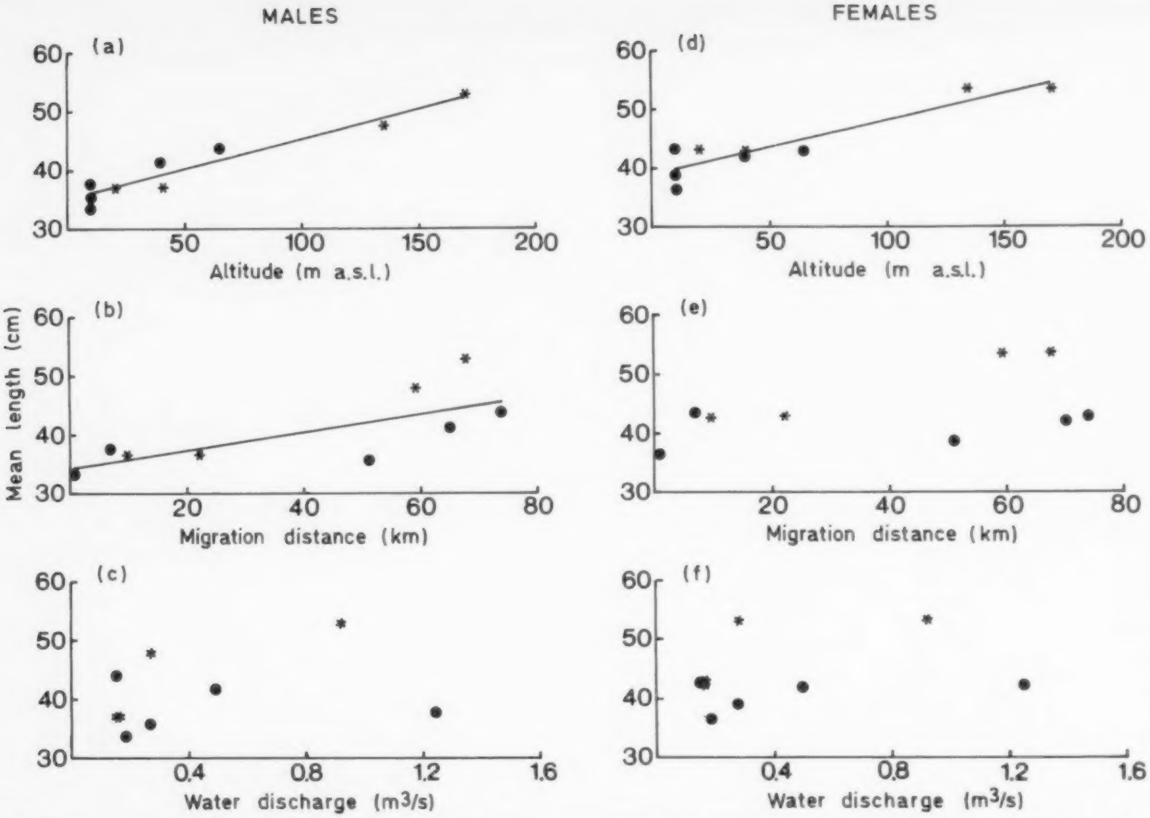

FIG. 2. Relationships between spawning site altitude, distance of freshwater migration, and mean water discharge during spawning and mean size of mature males and females for nine populations of anadromous brown trout in the River Gaula (asterisks) and the River Namsen (solid points).

TABLE 4. Linear regression statistics for the least-squares regression lines in Fig. 2 and 3 based on nine measurements of mean adult length (cm) and mean maturity sea age (summers at sea) as dependent variables (Y) and spawning site altitude (m a.s.l.), and migration distance in freshwater (km) as independent variables (X) yielded $Y = a + bX$; r = regression coefficient. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Group	*a*	*b*	*r*
Length vs. spawning site altitude			
Males	34.8	1.034	0.96***
Females	38.9	0.909	0.92***
Length vs. migration distance			
Males	34.3	1.546	0.73*
Age vs. spawning site altitude			
Males	2.2	0.0042	0.72*
Females	2.4	0.0042	0.81**
Age vs. migration distance			
Males	2.1	0.0080	0.70*
Females	2.3	0.0074	0.73*

utary, and it varied from 0.6 to 73.9 km. The brown trout spawned in the tributaries about 100–500 m from the main river. The altitudes of the spawning areas were estimated from topographic maps.

Statistical Analysis

Interriverine analysis of adult length and sea age at maturity were carried out with analysis of covariance (ANCOVA) only when the dependent and independent variables met the assumptions of this model. In this study, these assumptions were significant linearity of each relation between Y and X and homogeneity in slopes (Sokal and Rohlf 1981). Intrariverine analysis of traits were carried out with analysis of variance (ANOVA). Linear least-squares regression models were developed (SPSS/PC) for mean length and mean age at sexual maturity on spawning site altitude and migration distance.

Results

Length of Male and Female Fish

Females were generally larger than males but the difference was significant in only three populations (Table 3). The mean lengths of males increased significantly ($P < 0.05$) with increasing altitude and migration distance (Fig. 2a and 2b; Table 4), but not with mean water discharge in the streams (Fig. 2c). When computing the linear least-squares multiple regression of mean lengths of males on spawning site altitude and migration distance (the latter two variables were significantly uncorrelated (Pearson's $r = 0.619$, $n = 9$)), the latter did not significantly improve the coefficient of determination of mean lengths of males and spawning site altitude. This indicates that elevation was of major importance in determining length of male trout. Interriverine differences

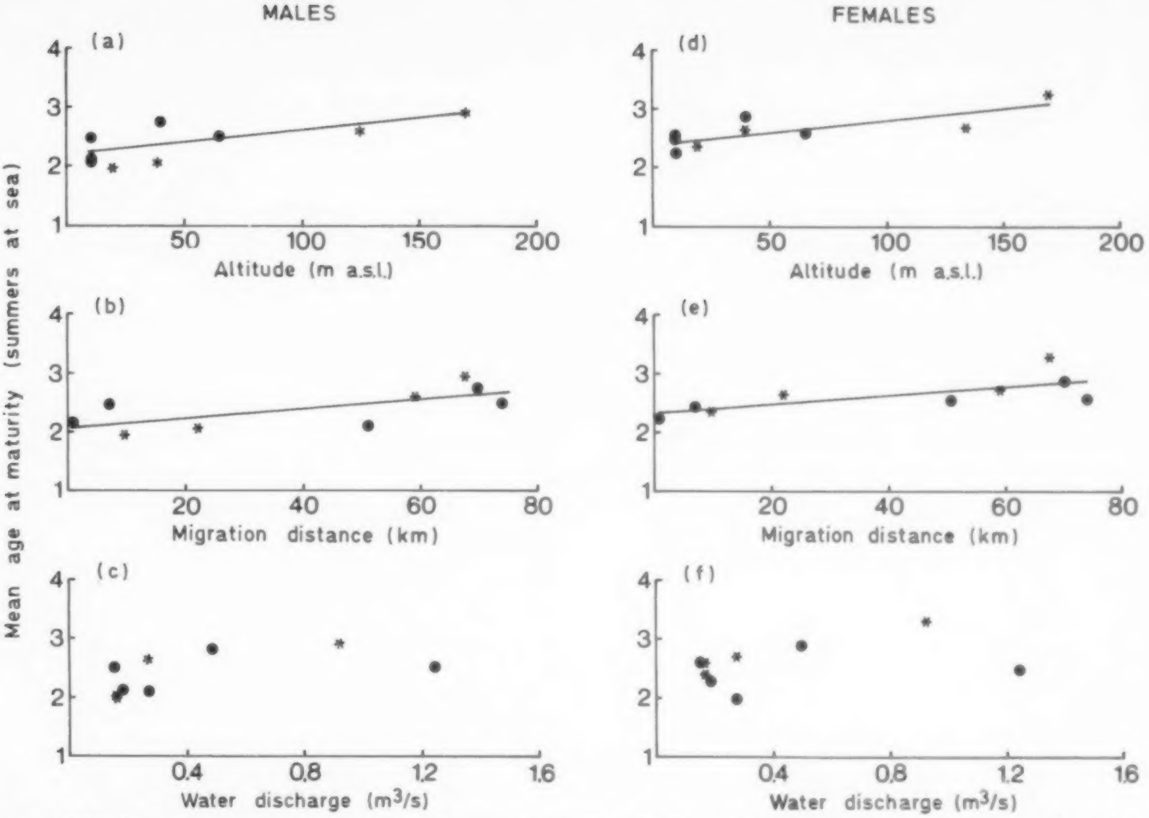

FIG. 3. Relationships between spawning site altitude, distance of freshwater migration, and mean water discharge during spawning and mean age at sexual maturity of males and females for nine populations of anadromous brown trout in the River Gaula (asterisks) and the River Namsen (solid points).

TABLE 5. Frequency (arithmetic means ± 95% C.L. and ranges) of redd grain sizes from four tributaries into the River Gaula.

		Grain size (mm)			
Tributary	*n*	<2.0	2.0–6.3	6.3–20.0	>20.0
1. Bortna	3	1.9 ± 4.47 (0.6–4.0)	4.5 ± 3.97 (3.3–6.3)	13.8 ± 8.45 (11.2–17.7)	79.9 ± 6.96 (77.9–83.1)
2. Loddbekken	7	6.1 ± 6.38 (0.2–14.6)	7.9 ± 4.81 (2.2–15.6)	21.0 ± 7.40 (12.1–33.8)	65.0 ± 15.91 (43.1–85.6)
3. Malma	3	7.7 ± 3.23 (6.4–8.9)	9.7 ± 3.73 (8.4–11.4)	23.3 ± 12.17 (19.3–28.8)	59.4 ± 17.14 (52.1–65.9)
4. Herjåa	2	1.2 ± 7.19 (0.6–1.7)	1.8 ± 0.90 (1.7–1.8)	7.1 ± 46.72 (3.4–10.8)	89.8 ± 35.94 (86.9–92.6)

did account for a significant portion of the variation in mean lengths of males after the effect of spawning site altitude was accounted for (ANCOVA, $P < 0.05$).

The mean lengths of females increased significantly ($P < 0.05$) with spawning site altitude (Fig. 2d; Table 4), but not with migration distance or water discharge (Fig. 2e and 2f). When computing the linear least-squares multiple regression of mean lengths of females on spawning site altitude and migration distance, the latter did not significantly improve the coefficient of determination of mean lengths of females and spawning site altitude. This indicates that elevation was of major importance in determining the length of female trout.

Analysis of the variability of fish length within the rivers showed that a significant degree of heterogeneity existed within sexes among the different populations ($P < 0.05$). Spawners of both sexes in the two uppermost tributaries of the River Gaula were significantly larger than those in the two lowermost tributaries (Scheffé procedure on means, $P < 0.05$). Males in the uppermost tributary in the River Namsen were significantly larger than the males of the populations in the three lowermost tributaries ($P < 0.05$), and males in Almåselven (population No. 4) were also significantly larger than males of the lowermost population. No two groups of females were significantly different ($P > 0.05$) in the River Namsen.

Age at Sexual Maturity

Females were generally sexually mature at an older sea age than males, but the difference was significant in only two populations (Table 3). The mean age at sexual maturity of males increased significantly ($P < 0.05$) with migration distance and spawning site altitude (Fig. 3a and 3b; Table 4), but not with water discharge (Fig. 3c). When computing the linear least-squares multiple regression of mean age of males on spawning site altitude and migration distance, the latter did not significantly improve the coefficient of determination of mean age of males and spawning site altitude. Interriverine difference was significant for male age at maturity when migration distance was a covariate (ANCOVA, $P < 0.05$).

The mean age at sexual maturity of females increased significantly ($P < 0.05$) with spawning site altitude and migration distance (Fig. 3d and 3e; Table 4), but not with water discharge (Fig. 3f). When computing the linear least-squares multiple regression of mean age of females on spawning site altitude and migration distance, the latter significantly improved the coefficient of determination from $R^2 = 0.648$ to $R^2 = 0.734$. Interriverine difference was significant for female age at maturity with migration distance as covariate (ANCOVA, $P < 0.05$).

The ages at maturity of both sexes (treated separately) among the populations in the River Gaula were significantly different (ANOVA, $P < 0.001$), but those in the River Namsen populations were not ($P > 0.05$). Comparison of means revealed that males and females of the two uppermost populations of the River Gaula matured sexually at a significant older age than those in the two lowermost populations ($P < 0.05$).

Grain Size of Redds

The variation in redd structure was approximately the same within as among tributaries (Table 5). The fraction >20.0 mm made up more than 50% of the gravels in the redds. The largest mean lengths and oldest sea age at maturity of brown trout populations were found in the tributaries with the lowest (Malma) and the highest (Herjåa) values of the largest grain fraction. This indicates that there is no selection for large brown trout to spawn on coarse gravel.

Discussion

For all the nine populations of anadromous brown trout studied, the length of the spawners and their sea age at maturity were significantly correlated with the migration distance and spawning site altitude. ANCOVA revealed that the interriverine differences accounted for only a minor part of the overall variation in these particular life-history traits. Interriverine effects were recorded only in mean length of males with spawning site altitude as the covariate and in sea age at maturity of males and females with migration distance as the covariate. Neither mean water discharge in the tributaries during spawning nor that in the main river (L'Abée-Lund et al. 1989) had any influence on these life-history traits. This contrasts with the observation made by Jonsson (1985) that adult brown trout size was related to the rate of water discharge of the tributary in which the fish spawned. Because the migration distance and spawning site altitudes were identical for all three of Jonsson's (1985) populations, his study provided a more appropriate test for the significance of water discharge alone.

Differences in fish length among populations could be due to differences in size of gravel substrate, as the suitability of a particular size gravel substrate depends mostly on fish size (Reiser and Bjornn 1979). However, analysis of the mean grain size of brown trout redds in four streams into the River Gaula revealed no relationship with mean length of female brown trout. This is in accordance with a more detailed study on the structure of brown trout redds (Ottaway et al. 1981). Thus, the size of gravel substrate for spawning is of minor importance in explaining interpopulation variation in mean brown trout size.

Second, in the present study, the uppermost populations in both rivers have to overcome stretches of rapids before reaching their natal areas to spawn. However, the two lowermost populations in both rivers encounter no rapids during their migration from the sea to their natal tributaries. This implies that the energy requirement during the spawning migration will be greater for the populations spawning further upriver than that for the downriver populations. An increased energy requirement can be compensated for by an increase in body size, since power loading and body size are positively correlated (Bonner 1965). Glebe and Leggett (1981) showed that the energy cost of migration was higher in small American shad than in larger ones. An intraspecific variation in body size of the dark-eyed junco, *Junco hyemalis* (Ketterson and Nolan 1976), and of the red deer, *Cervus elaphus* (Langvatn and Albon 1986), has been found to be positively correlated with the distance of migration. Studies of the energetics of migration of the American shad and of several salmonid species have shown that a large body size provided a greater range of those swimming speeds over which the absolute energy requirement was minimal (Weaver 1963; Leggett and Trump 1978). Thus, energy requirement is probably a factor selecting for larger sized individuals in upriver fish populations. Because fish growth decreases after sexual maturity (Nikolsky 1963), the fish achieves larger length by delaying sexual maturity. This was observed in the present study. In the populations with the largest individuals, these individuals attained sexual maturity at an older age than fish in the smaller sized populations. This suggests that environmental severity might have a considerable influence on certain life-history traits of anadromous brown trout populations. In coregonines, freshwater migration is also an important factor, responsible for the different reproductive patterns exhibited by anadromous species (Lambert and Dodson 1990a, 1990b).

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References

BONNER, J. T. 1965. Size and cycle. An essay on the structure of biology. Princeton University Press, Princeton, NJ. 219 p.

CARSCADDEN, J. E., AND W. C. LEGGETT. 1975. Life history variations in populations of American shad, *Alosa sapidissima* (Wilson), spawning in tributaries of the St. John River, New Brunswick. J. Fish Biol. 7: 595–609.

DAHL, K. 1910. Alder og vekst hos laks og ørret belyst ved studier av deres skjæl. Landbruksdepartementet, Kristiania. 115 p. (In Norwegian)

1917. Studier og forsøk over ørret og ørretvand. Centraltrykkeriet, Kristiania. 107 p. (In Norwegian)

FLEMING, I.A., AND M. R. GROSS. 1989. Evolution of adult female life history and morphology in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43: 141–157.

GLEBE, B. D., AND W. C. LEGGETT. 1981. Latitudinal differences in energy allocation and use during freshwater migrations of American shad (*Alosa sapidissima*) and their life history consequences. Can. J. Fish. Aquat. Sci. 38: 806–820.

HALVORSEN, M., AND O. B. STABELL. 1990. Homing behaviour of displaced stream-dwelling brown trout. Anim. Behav. 39: 1089–1097.

HINDAR, K., B. JONSSON, N. RYMAN, AND G. STÅHL. 1991. Genetic relationships among landlocked, residents, and anadromous brown trout, *Salmo trutta* L. Heredity 66: 83–91.

JONSSON, B. 1985. Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Trans. Am. Fish. Soc. 114: 182–194.

Ketterson, E. D., and V. Nolan Jr. 1976. Geographic variation and its climatic correlates in the sex ratio of eastern-wintering dark-eyed juncos (*Junco hyemalis hyemalis*). Ecology 57: 679–693.

L'Abée-Lund, J. H., B. Jonsson, A. J. Jensen, L. M. Sættem, T. G. Heggberget, B. O. Johnsen, and T. F. Næsje. 1989. Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. J. Anim. Ecol. 58: 525–542.

Lambert, Y., and J. J. Dodson. 1990a. Freshwater migration as a determinant factor in the somatic cost of reproduction of two anadromous coregonines of James Bay. Can. J. Fish. Aquat. Sci. 47: 318–334.

1990b. Influence of freshwater migration on the reproductive patterns of anadromous populations of cisco (*Coregonus artedii*) and lake whitefish (*C. clupeaformis*). Can. J. Fish. Aquat. Sci. 47: 335–345.

Langvatn, R., and S. D. Albon. 1986. Geographic clines in body weight of Norwegian red deer: a novel explanation of Bergmann's rule? Holarct. Ecol. 9: 285–293.

Leggett, W. C., and C. J. Trump. 1978. Energetics of migration in American shad, p. 370–377. *In* K. Schmidt-Koenig and W. T. Keeton [ed.] Animal migration, navigation, and homing. Springer-Verlag, Berlin.

Mayr, E. 1970. Populations, species, and evolution. The Belknap Press, Cambridge, MA. 453 p.

Nikolsky, G. V. 1963. The ecology of fishes. Academic Press, London. 352 p.

Ottaway, E. M., P. A. Carling, A. Clarke, and N. A. Reader. 1981. Observations on the structure of brown trout, *Salmo trutta* Linnaeus, redds. J. Fish Biol. 19: 593–607.

Reiser, D. W., and T. C. Bjornn. 1979. Habitat requirements of anadromous salmonids, p. 1–54. *In* W. R. Meehan [ed.] Influence of forest and rangeland management on anadromous fish habitat in Western North America. U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. PNM-96, Portland, OR.

Scarnecchia, D. L. 1983. Age at sexual maturity in Icelandic stocks of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 40: 1456–1468.

Schaffer, W. M., and P. F. Elson. 1975. The adapative significance of variations in life history among local populations of Atlantic salmon in North America. Ecology 56: 577–590.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco, CA. 859 p.

Stuart, T. A. 1957. The migrations and homing behaviour of brown trout (*Salmo trutta* L.). Freshwater Salmon Fish. Res. 18: 1–27.

Thorpe, J. E., and K. A. Mitchell. 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness, and current management. Can. J. Fish. Aquat. Sci. 38: 1576–1590.

Weaver, C. R. 1963. Influence of water velocity upon orientation and performance of adult migrating salmonids. U.S. Dep. Interior Fish. Bull. 63: 97–121.

Response of a Phytoplankton Community to Controlled Partial Recovery from Experimental Acidification

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Lake 223 was experimentally acidified with H_2SO_4 from 1976 to 1989. The pH was reduced from 6.7 (1974) to 5.0 (1981) and held there for 3 yr (1981–83). Beginning in 1984 the pH was allowed to increase at a controlled rate by reduction of acid additions, resulting in a two-step recovery of 4 yr at pH ~5.5 and 2 yr at pH ~5.8. During the first 6 yr of early recovery, species diversity of the phytoplankton community increased linearly with pH and the number of common species increased. Total phytoplankton biomass remained elevated above preacidification estimates or increased. Dinoflagellates and cyanophytes remained codominant with little evidence of other taxonomic groups increasing.

De 1976 à 1989, le lac 223 a été artificiellement acidifié. Le pH est passé de 6,7 en 1974 à 5,0 en 1981; il a été maintenu à ce niveau pendant 3 ans, soit de 1981 à 1983. En 1984, le pH a été graduellement augmenté à un taux contrôlé par réduction des ajouts d'acide pour obtenir un rétablissement du pH en deux étapes, soit 4 ans à un pH d'environ 5,5 et 2 ans à un pH d'environ 5,8. Au cours des six premières années du rétablissement, la diversité des espèces de la communauté phytoplanctonique a augmenté de façon linéaire en fonction d'une augmentation du pH et le nombre d'espèces communes a augmenté. La biomasse totale de phytoplancton est demeurée supérieure aux estimations effectuées avant l'acidification ou a augmenté. Les dinoflagellés et les cyanophycées sont demeurés codominants et les autres groupes taxonomiques ne se sont pas révélés plus abondants.

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Acid precipitation caused by sulfur and nitrogen emissions continues to be a major perturbation of freshwater ecosystems. The only available literature on phytoplankton population responses to recovery from acidification are paleolimnological studies (Dixit et al. 1989) and experiments where a technique referred to as "liming" has been applied (Scheider et al. 1976; Yan and Dillon 1984; Hultberg and Andersson 1982). After liming two lakes in the Sudbury area, Scheider et al. (1976) documented an immediate increase in biomass and a shift in dominance to chrysophytes. However, Hultberg and Andersson (1982) documented a slow recovery response over 5 yr for four limed lakes in Sweden. Neither of these studies had preacidification data against which to compare recovery results. In this study, a whole-lake acidification experiment was begun in 1976 on Lake 223 at the Experimental Lakes Area (ELA) (Schindler et al. 1980, 1985; Findlay and Kasian 1986). The phytoplankton community was monitored intensively for 2 yr prior to acidification (1974–75) and for 14 consecutive yr during acidification.

The pH in Lake 223 was reduced gradually from 6.7 (1974) to ~5.0 (1981–83) by epilimnetic additions of H_2SO_4. The responses of the Lake 223 epilimnetic phytoplankton community to decreasing pH (Findlay 1984; Findlay and Kasian 1986, 1990) were similar to those observed in other studies involving both enclosure experiments and atmospherically acidified lakes (Almer et al. 1978; Kwiatkowski and Roff 1976; Yan and Stokes (1978). In its natural state (1974–75) the phytoplankton community in Lake 223 was comparable with other natural systems in the ELA (Findlay and Kasian 1990). Species diversity was high with a chrysophycean dominated assemblage accompanied by smaller populations of diatoms, chlorophytes, and cryptophytes. Biomass at this time averaged 650 $g \cdot m^{-3}$, which was typical for lakes in this area. Decreasing pH caused a shift in species composition to a predominance of dinoflagellates and cyanophytes. Species diversity decreased by 30%, phytoplankton biomass increased by 40%, and the portion of phytoplankton biomass <20 μm in size increased up to two times.

This paper presents the response of the phytoplankton community to the first 6 yr of controlled partial recovery of pH from 5.0 to 5.8 in experimentally acidified Lake 223. In 1984, recovery of Lake 223 was initiated with the intent of eventually allowing pH to rebound to a natural circumneutral level. In the early stage, pH was allowed to increase at a controlled rate by reduction of acid additions, resulting in a two-step recovery of 4 yr at pH ~5.5 and 2 yr at pH ~5.8. This is in contrast with the previously mentioned liming experiments where the pH was forced from acidic levels below 5 to levels well above pH 6 within short time spans.

The phytoplankton community composition in Lake 223 had responded quickly once pH declined below pH 5.6 during the acidification phase. A similar response was expected when pH rose above 5.6 during recovery because rapid responses in the phytoplankton community composition had been documented when nutrient additions were terminated in Lake 226 North and South and in Lake 304 (Schindler 1974; Findlay and Kasian 1987). In these lakes the phytoplankton community composition recovered immediately. As well, Dixit et al. (1989) documented the rapid response in Swan Lake (Ontario) to recovery

TABLE 1. Mean annual ice-free season pH (time weighted), suspended P/C ratio, suspended N/C ratio, total phosphorus, total phytoplankton biomass, and size fractions of phytoplankton biomass for Lake 223 during 1974–89.

Year	pH	Suspended P/C ($\mu g \cdot mg^{-1}$)	Suspended N/C ($\mu g \cdot mg^{-1}$)	Total phosphorus ($\mu g \cdot L^{-1}$)	Phytoplankton biomass ($mg \cdot m^{-3}$)	Size fractions of phytoplankton biomass ($mg \cdot m^{-3}$) <20 μm	<40 μm	>40 μm
				Preacidification				
1974	6.7	7.1[a]	120[a]	7.0	683	407	565	118
1975	6.6	5.0	80	6.5	647	348	516	132
				Acidification				
1976	6.5	4.6	100	5.2	901	560	732	169
1977	6.1	4.8	120	6.3	1078	578	801	277
1978	5.9	6.3	100	5.9	1091	651	926	165
1979	5.6	5.6	120	5.2	907	482	708	199
1980	5.6	5.9	60	5.3	1969	980	1428	542
1981	5.0	6.8	80	6.2	1620	643	774	847
1982	5.1	6.9	80	6.8	1364	482	630	735
1983	5.1	8.4	80	6.4	1505	556	872	634
				Recovery				
1984	5.4	8.7	180	10.5	1789	318	1184	605
1985	5.5	8.4	110	9.4	1125	485	763	363
1986	5.5	6.2	90	6.2	1366	592	875	491
1987	5.4	6.1	100	6.1	1537	615	783	755
1988	5.8	5.6	100	5.8	1430	495	691	740
1989	5.8	5.4	90	5.5	1991	769	975	1017

[a]Ice-free annual average is based on six samples as compared with a minimum of 12 for all other years.

from acidification using paleolimnological techniques based on chrysophyte scales. A rapid response in Lake 223 might be expected because of the rapid turnover time of the phytoplankton populations and access to reinvasion by populations from upstream Lake 224. Therefore, for Lake 223 recovery, we hypothesized a rapid decrease in dinoflagellates with a concurrent increase in chrysophytes as the pH rose above 5.6. Because biomass increased as pH decreased during the acidification phase, we also hypothesized that biomass would decrease during recovery. Species diversity decreased in Lake 223 during acidification because of the predominance of a few large acid-tolerant dinoflagellate species. We hypothesized that increased pH would allow a wider diversity of species to exist.

Methods

Study Site and Experimental Design

The ELA, northwestern Ontario, is situated on Precambrian gneiss and granites and the lake watersheds are surrounded by typical boreal pine and spruce forests. Lake 223 is a dimictic lake located in the ELA. It has a surface area of 27 ha and a maximum depth of 14 m. It is naturally oligotrophic, with an inflowing stream from Lake 224, and has a single outlet. Prior to manipulation the chemical regime was typical for lakes in this area (Schindler et al. 1980). In 1974–75, average suspended carbon was 605 $\mu g \cdot L^{-1}$, pH was 6.74, and conductivity was 20.7 $\mu S \cdot cm^{-1}$ as reported by Prokopowich (1979). Epilimnetic pH in Lake 223 was reduced from 6.7 to 5.0 (1976–81) and was held at pH 5.0 from 1981 to 1983 by additions of H_2SO_4 (Cruikshank 1984). From 1984 to 1989, pH was allowed to increase to predetermined annual levels (Table 1) by reductions in the volume of acid additions (Cruikshank 1986).

Reference lakes chosen for this experiment were 224 and 239 which are also oligotrophic, chemically typical of the ELA (Armstrong and Schindler 1971; Prokopowich 1979), and unmanipulated. They are 25 and 56 ha in area and have maximum depths of 25 and 30 m, respectively (Cleugh and Hauser 1971). In a previous paper dealing with the acidification phase (Findlay and Kasian 1986), five reference lakes were used for comparison. Three of those lakes have since been subjected to experimental manipulation and therefore can not be used in the recovery study.

Sampling and Enumeration

This is a continuation of the acidification study described by Findlay and Kasian (1986), and methodology is identical to that described in the previous paper. Phytoplankton samples were taken every 2 wk through the ice-free season using an integrating sampler (Shearer 1978) to obtain samples for the epilimnion. A 10-mL aliquot of Lugol's preserved sample was settled for 24 h. Phytoplankton species were identified and enumerated using an inverted microscope applying the Utermöhl technique (Nauwerck 1963). Cell density for each species was converted to wet weight biomass by measuring individual cells of each species, applying the geometric formula best fitted to the cell shape (Vollenweider 1968), and assuming a specific gravity of 1 for the cellular mass. Cell sizes were regularly measured and averages were calculated from up to 50 individuals of each species.

Diversity

Simpson's diversity index was chosen to quantify changes in species numbers relative to cell abundance. Washington

(1984) concluded that this was the best index available for aquatic populations even though it was biased towards dominant species. This index is

$$D = 1 - \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{n(n-1)}$$

where S = the number of species in a "sample", n = the number of individuals in a sample, and n_i = the number of individuals of species i in a sample. Species diversity (D) would be 1 in a homogeneous community where each species contributed equally to the total abundance and would be near 0 in a community with the same composition but dominated by a few species.

This index should accurately reflect species diversity in these samples. The volume of sample counted was constant throughout the study. The median number of cells counted per sample was 2300. For the 192 samples counted, there was no significant correlation between the number of cells counted and the number of species identified ($r^2 < 0.0001, p = 0.91$). As well, this index is relatively insensitive to the number of rare species in a sample.

Size Fractions of Biomass

To examine structural changes in the phytoplankton assemblage which could impact food web interactions, species were grouped into three size fractions (<20, <40, and >40 μm in any dimension).

Statistical Comparison with Reference Lakes

The phytoplankton population of Lake 223 was compared with the populations in two reference lakes (224 and 239) to separate experimental responses from temporal trends. A repeated measures, nested design analysis of variance (ANOVA) in the general linear models (GLM) procedure in the SAS (SAS Institute Inc. 1985) statistical package was performed on annual means of specified parameters. Annual means for each lake were calculated from 12–16 biweekly samples during the ice-free season for each year. The ANOVA was used to test two hypotheses: P_t is the probability that the long-term trend in Lake 223 was equal to the average long-term trend in reference lakes; P_m is the probability that the overall mean in Lake 223 is equal to the overall mean in reference lakes. It should be noted that the ANOVA used here was less sensitive in testing these hypotheses than in Findlay and Kasian (1986) because the decrease from five to two reference lakes reduced degrees of freedom in the error terms, especially for mean levels (P_m) tests.

Average linkage clustering (Proc Cluster in SAS) was used to compare phytoplankton species composition in Lake 223 throughout the acidification and partial recovery experiment with that in the reference lakes in the same years. The analysis was done on the percent contribution of each species to the total annual ice-free season biomass. Because cluster analysis is sensitive to a high proportion of zeros in the matrix, a reduced list of 62 species was compiled from the common species to include those making up 75% of total biomass in each year for Lakes 223, 224, and 239. The list was further reduced to 41 species by removing any species which occurred only once in the 46 lake-years and which had a biomass contribution of less than 3%. Average linkage clustering was performed on this 41 × 46 species by lake-year matrix. Examination of species means for the five major resulting clusters revealed that 13 species did not significantly contribute to differentiating these clusters. Average linkage clustering was performed on the reduced 28 × 46 species by lake-year matrix, and the same five major clusters were recovered.

Results

During the first 6 yr of recovery of Lake 223, as pH increased from 5.1 to 5.8, there was little change in the contributions of major taxonomic groups or seasonal successions established during acidification (Fig. 1). Dinoflagellates and cyanophytes continued to codominate phytoplankton assemblages during stratified seasons, with substantial populations of chrysophytes occurring in spring and fall. In contrast, the phytoplankton assemblage in Lake 223 during the preacidification period and in reference lakes 239 and 224 during the 16-yr period were dominated by chrysophytes throughout the entire ice-free season (Fig. 1). Phytoplankton species in Lake 223 which continued to dominate the assemblage are those that were dominant at pHs around 5.0, such as *Gymnodinium* sp., *Peridinium inconspicuum* Lemm., *Chroococcus minutus* (Kütz.) Naegeli, and *Merismopedia minima* Beck with notable increases in *Peridinium limbatum* (Stokes) Lemm., *Peridinium Willie* Huitfelt-Kaas, and *Rhabdogloea linearis* Sch. and Laut.

Figure 2 shows the dendrogram which resulted from performing average linkage clustering on the 28 × 46 species by lake-year matrix. The cubic clustering criterion indicated a significant grouping at the five-cluster level. Cluster means for each of the 28 species are shown in Fig. 3. Preacidification years 1974–75 and 1976 for Lake 223 fall in a tight cluster (C1) with the same years for reference lakes 224 and 239 (Fig. 2). C1 is separated from the larger reference lake cluster C2 because of larger average percentages of small and large chrysophycean groups. From 1976 to 1989, more effort was made to identify species contributing to these groupings. After 1976, *Chrysochromulina parva* Lackey, *Uroglena americana* Calk., and *Stichogloea Doederleinii* (Sch.) Wille were identified to species level. To test whether 1974–76 were outliers from the reference group because of taxonomic resolution, another clustering analysis was performed in which *Uroglena americana* and *Stichogloea Doederleinii* were returned to the large chrysophycean grouping and *Chrysochromulina parva* to the small chrysophyte grouping for all 46 lake-years. C1 and C2 from the original dendrogram (Fig. 2) formed one large cluster, but a significant degree of sensitivity was lost in differentiating acidification and recovery years for Lake 223.

C2 in Fig. 2 is characterized by large percentages of *Uroglena americana*, *Dinobryon sertularia* Ehrenb., *Dinobryon bavaricum* Imhof, large chrysophytes, and small chrysophytes (Fig. 3). The percentage of cyanophytes is also low. The diatoms *Asterionella ralfsii* var. *americana* Körn. and *Rhizosolenia eriensis* H. L. Smith are moderately abundant. Most years for Lake 224 form a fairly tight group within C2 (Fig. 2). However, Lake 239 shows more variation, with single or paired years joining the overall cluster relatively late. Three early years of acidification (1977–79) in Lake 223, when pH had not decreased significantly, also fall within C2. Lake 223 in 1985 also falls within this reference lake cluster and is shown to be most similar in phytoplankton composition to Lake 223 in 1979. In 1985, above-normal levels of precipitation made it difficult to regulate pH. As a result the pH range within the year was

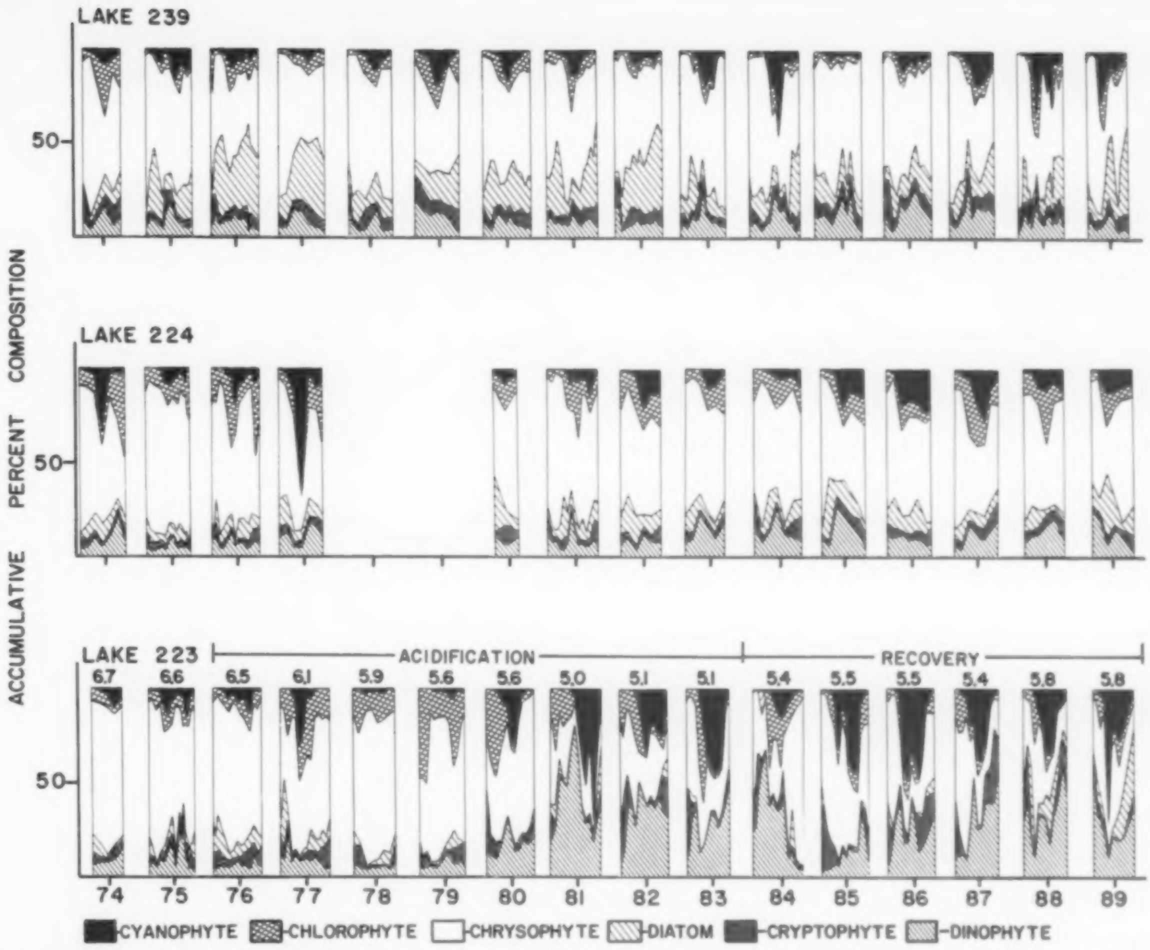

FIG. 1. Accumulative percent composition of phytoplankton for experimentally acidified Lake 223 and reference lakes 224 and 239 for 1974–89. The mean annual, time-weighted pHs are listed at the top of the panel for Lake 223.

larger, and possibly allowed an increase in chrysophytes (Fig. 1).

C5 is composed of only 1981 and 1982 when pH was ~5.0 in Lake 223. This cluster is most different of the five and is the furthest distance from C2 (Fig. 2). These 2 yr have a mean composition of 37% *Gymnodinium* sp., 14% *Chroococcus minutus*, and virtually no chrysophytes. Diatoms and cryptophytes were also absent.

Recovery years, along with 1980 and 1983, fall into C3. This cluster is different from C2 in having a high percentage of *Gymnodinium* sp. along with *Peridinium Willie* and *Peridinium limbatum* and consistently higher percentages of cyanophytes. Overall chrysophyte abundance is down, with a shift from *Dinobryon sertularia* and *Uroglena americana* to *Stichogloea Doederleinii*. Species shifts occurred within the cryptophytes and chlorophytes. *Gonyostomum semen* (Ehrenb.) Diesing replaced *Rhodomonas minuta* Sch. and Laut., and *Gloeococcus Schroeteri* (Chod.) Lemm. replaced *Botryococcus Braunii* Kütz.

C4 is made up of a single acidified year, 1984. High percentages of *Dinobryon sertularia* distinguished it from other acidification or recovery years.

Phytoplankton biomass in Lake 223 during early recovery (1984–89) was greater than during preacidification years and was also higher than in reference lakes (Fig. 4a). ANOVA was not able to detect a significant difference between Lake 223 and reference lakes because of large confidence intervals during the recovery period (Table 2). Continued high biomass was due primarily to continued high abundances of dinoflagellates and cyanophytes (Fig. 4b, 4c). Continually elevated biomass throughout acidification and early recovery years was not directly correlated with total phosphorus ($r^2 = 0.026$, $p = 0.57$) (Table 1). This is not unexpected in light of the observations of Healey (1973), who documented decreased phosphorus efficiency of algae below pH 6.5. During recovery, chrysophycean abundance was similar to the trend and mean abundance observed in reference lakes (Fig. 5a). Significantly different trends were detected from reference lakes for chlorophytes and diatoms in Lake 223, but mean abundances of these two groups were well within the range of reference lakes (Table 2). Chlorophycean abundance deviated from the reference trend in 1989, while diatoms reacted similarly in 1988 (Fig. 5b, 5c).

Decreasing pH in Lake 223 caused phytoplankton species diversity to decrease (Findlay and Kasian 1986). Phytoplankton

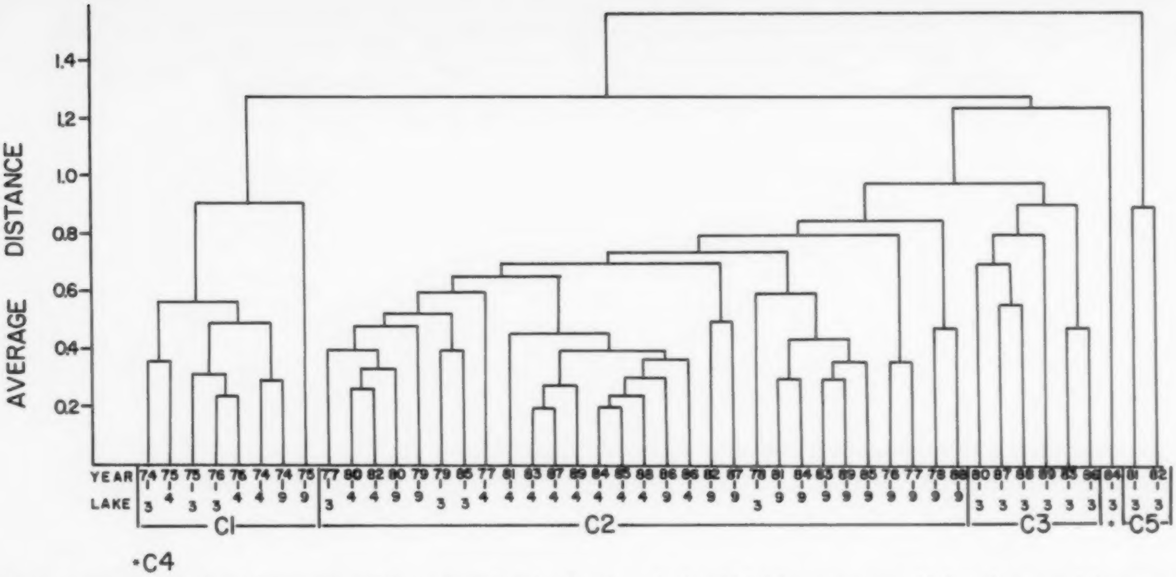

FIG. 2. Dendrogram produced from average linkage clustering showing the order in which lake-years were added to clusters and the average distance between clusters as they were formed. The groupings at the five-cluster level (C1–C5) are indicated at the bottom by boxes. Lake codes: 3 = Lake 223, 4 = Lake 224, and 9 = Lake 239.

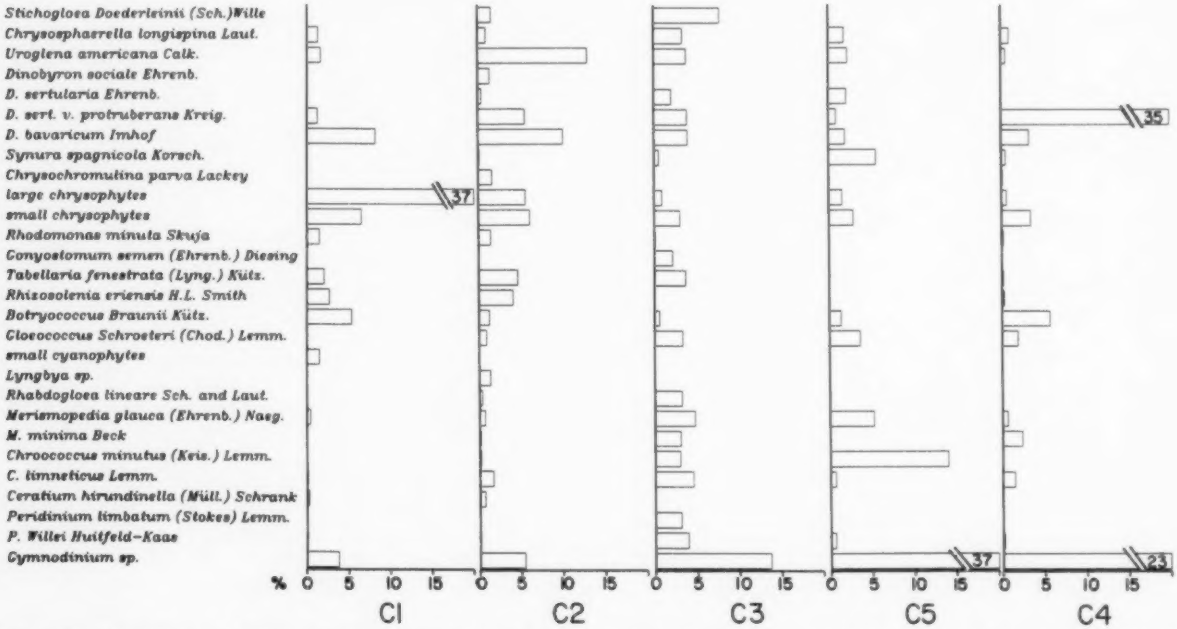

FIG. 3. Plot of each of the 28 species used in the analysis depicting the mean percent abundance of the lake-years in each of the five clusters indicated in Fig. 2.

species diversity increased as pH increased during the recovery period, but it remained below the mean diversity of the reference lakes (Fig. 6a). There was a significant correlation between phytoplankton species diversity and pH in Lake 223 ($r^2 = 0.69$, $p = 0.0004$) (Fig. 6b). Increased diversity can be attributed to an increase in the number of common species (those species constituting 50–90% of the biomass) (Fig. 7). The number of common species remained low during the first year of recovery (pH 5.4). Once pH increased above pH 5.5, the total number of species recorded annually remained constant and the number of common species increased.

No significant difference in the trend or mean for size fractions of phytoplankton biomass <20 and <40 μm was detected between the reference lakes and Lake 223 during early recovery

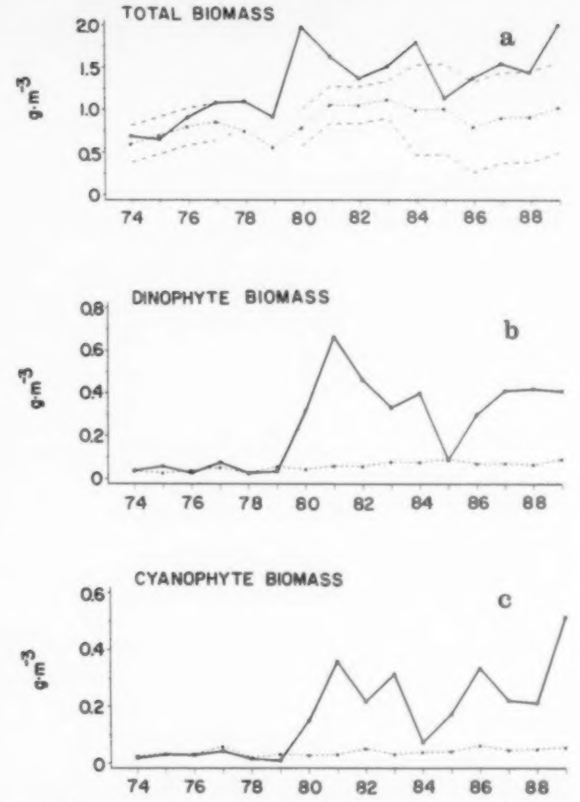

FIG. 4. Results of nested ANOVA comparing Lake 223 with the mean of the reference lakes for 1974–89. *, Lake 223; ●, mean of reference lakes 224 and 239. The broken lines are ±2 SD from the reference mean. Note that there are no confidence intervals for 1978 and 1979 because Lake 224 was not sampled in these years. (a) Total phytoplankton biomass; (b) dinoflagellate biomass (±2 SD not shown because they are too tight to the mean); (c) cyanophyte biomass (±2 SD not shown because they are too tight to the mean).

TABLE 2. Probability that the trend in Lake 223 during 1974–83 and 1984–89 is equal to the trend in the reference lakes for the same years. P_t is the probability that the long-term trend in Lake 223 was equal to the average long-term trend in the reference lakes; P_m is the probability that the overall mean in Lake 223 was equal to the overall mean in the reference lakes. An asterisk indicates a significant response.

	Acidification (1974–83)		Recovery (1984–89)	
Parameter	P_t	P_m	P_t	P_m
Biomass	0.04*	0.21	0.30	0.32
Diversity	0.03*	0.10	0.18	0.08*
Size fractions of biomass				
<20 μm	0.04*	0.08	0.59	0.54
<40 μm	0.03*	0.04*	0.49	0.65
>40 μm	0.01*	0.37	0.01*	0.05*
Cyanophyceae	0.001*	0.05*	0.003*	0.005*
Chlorophyceae	0.003*	0.28	0.04*	0.21
Chrysophyceae	0.18	0.24	0.45	0.91
Diatom	0.83	0.61	0.01*	0.76
Dinoflagellates	0.001*	0.002*	0.01*	0.03*

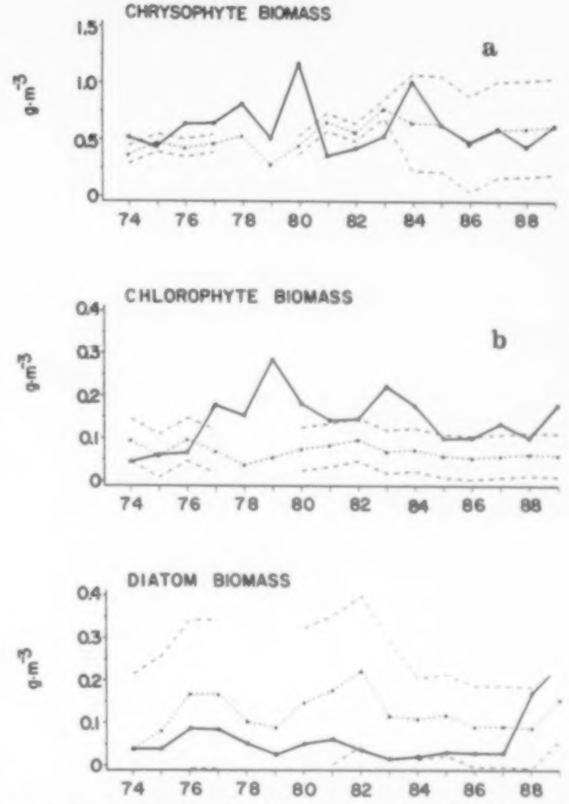

FIG. 5. Results of nested ANOVA comparing Lake 223 with the mean of the reference lakes for 1974–89. *, Lake 223; ●, mean of reference lakes 224 and 239. The broken lines are ±2 SD from the reference mean. Note that there are no confidence intervals for 1978 and 1979 because Lake 224 was not sampled in these years. (a) Chrysophyte biomass; (b) chlorophyte biomass; (c) diatom biomass.

(Table 2). However, the size fraction of biomass >40 μm remained elevated and steadily increased throughout recovery in Lake 223 (Table 2).

Discussion

There is conflicting evidence about the beginning of recovery from acidification in the phytoplankton community in Lake 223. Quantitative parameters such as phytoplankton species diversity or the number of common species increased, approaching levels seen in early acidification years. Total biomass remained elevated above the preacidification state, while total number of species exhibited no change. Qualitative parameters describing phytoplankton composition and succession indicated a lack of recovery.

As hypothesized, species diversity and the number of common species both increased during recovery. As pH increased towards neutrality the pool of species which existed also increased because there were acidophilic and circumneutral species coexisting at moderate pH levels.

From our experience with whole-lake eutrophication recovery experiments at the ELA, we assumed that composition and biomass would respond immediately to increasing pH. In Lakes 226 North, 226 South, and 304, dominance reverted back to chrysophytes and biomass decreased after treatments were ter-

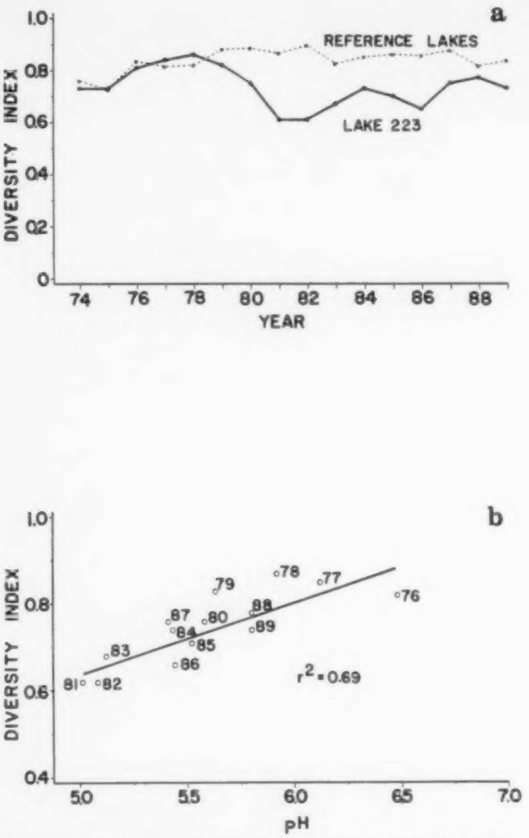

FIG. 6. Results of nested ANOVA comparing Lake 223 with the mean of the reference lakes for 1974–89. (a) Species diversity (Simpson's index) for Lake 223 versus the reference lakes from 1974 to 1989. *, Lake 223; •, mean of reference lakes 224 and 239. The ±2 SD intervals were too tight to plot. (b) Linear regression of diversity versus pH for Lake 223 from 1976 to 1989.

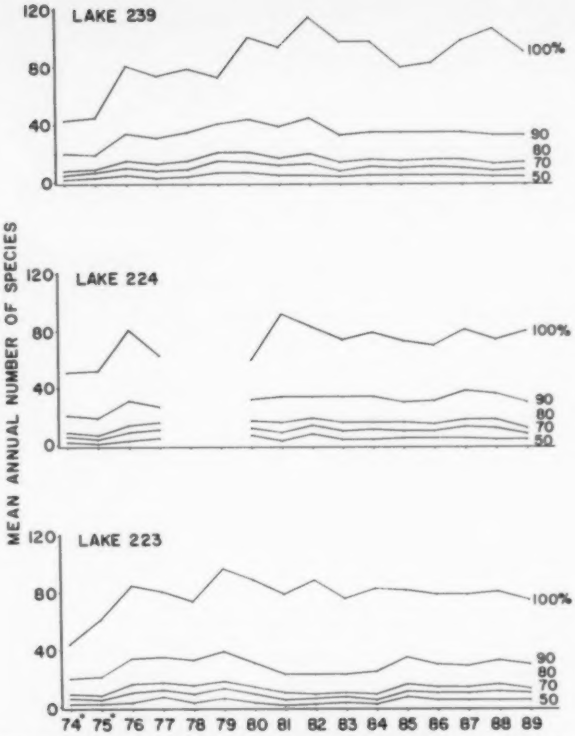

FIG. 7. Mean annual number of species representing 50, 70, 80, 90, and 100% of the biomass in experimentally acidified Lake 223 and reference lakes 224 and 239 from 1974 to 1989. The asterisk on 1974 and 1975 denotes the fact that less effort was applied in taxonomic identifications in those years and therefore the number of species is lower.

minated (Schindler 1974; Findlay and Kasian 1987). Based on liming experiments, Hultberg and Andersson (1982) documented successful reclamation of four Swedish lakes over a 5- to 7-yr period (1974–80) when pH was maintained above 6. In the first year following liming, they observed an immediate decrease in phytoplankton biomass. However, recovery of the phytoplankton composition and succession to a preacidification assemblage occurred only after pH was maintained above 6 for the 5–7 yr of the experiment. During the early years of their experiment, dinoflagellates continued to dominate, although successive species shifts occurred within the group. Diatoms, chlorophytes, and small chrysophytes gradually increased to dominance over the 5 yr. For two lakes in the Sudbury area, Scheider et al. (1976) documented rapid responses to liming which increased these lakes to circumneutral pH levels. After the initial liming addition, biomass decreased but then increased dramatically in the following year, with composition reverting to an assemblage dominated by chrysophytes.

We suspect that differences in the two liming experiments compared with Lake 223 are due to additions of the liming agents. Hydrated lime is an algicide causing rapid decreases in total CO_2 and increases in pH (Whipple 1948, cited in Waters 1956). In the Sudbury lakes (Scheider et al. 1976) the lime additions were dissolved, concentrated slurry mixes added directly to the epilimnion at several intervals causing a decrease in biomass to the extent that chrysophytes regained the competitive advantage. The Swedish lakes (Hultberg and Andersson 1982) were limed around the shoreline, at various depths, and over the 5 yr period only 60–80% was actually dissolved. Liming occurred over the 5 yr at a lower rate than that achieved in the Sudbury experiments, causing a continual decrease in biomass but initially not enough to allow a competitive advantage to other groups. Although Lake 223 was not limed, pH has slowly increased to 5.8 (1 pH unit below the natural state), well below the neutral level attained in the Swedish lakes experiment, which was conducive to reclamation of the phytoplankton population. In Lake 223, a phytoplankton assemblage created by lowering the lake's pH to 5.0 persisted when pH was raised to 5.8. The stratified season was dominated by dinoflagellates and large populations of cyanophytes. Phytoplankton biomass remained elevated.

The compositional structure that persisted in Lake 223 was due to competition and not to a lack of a reinvasion population or changes in major nutrients. Lake 223 is stream fed by Lake 224 at an annual discharge rate of 82 000 $m^3 \cdot yr^{-1}$. Lake 224 has a phytoplankton population similar to that which existed in Lake 223 prior to acidification (Fig. 1, 2). Therefore, species that were previously common or dominant have been constantly invading Lake 223 from Lake 224 via the inflow stream but have failed to outcompete the more robust dinoflagellate and cyanophycean species. Changes in major nutrients did not affect phytoplankton biomass in Lake 223. In 1984 and 1985, total phosphorus was at a maximum, but expected increases in phytoplankton biomass did not occur in both years. Overall there was no correlation ($r^2 = 0.03$, $p = 0.58$) between phosphorus and biomass during acidification. This lack of correlation is likely due to a decreased phosphorus utilization efficiency by algae (Healey 1973). Based on Healey's (1975) detection limits for algal nutrient status, Lake 223 progressed from extreme (P/C < 5) to moderate (P/C 5–10) levels of phosphorus deficiency (Table 1). A similar situation occurs for the suspended N/C ratio, negating nitrogen as being a major controlling nutrient.

During the early stages of recovery, the larger size fraction of phytoplankton (>40 μm) has remained elevated or increased. This group is composed of several species of large silicious dinoflagellates and some colonial chrysophytes. The combination of size and palatability of these dominant species may affect interactions between phytoplankton and zooplankton populations in Lake 223. Further investigations are required to resolve these interactions.

Conclusion

We conclude that species diversity of the phytoplankton community in Lake 223 has begun to recover at pH 5.5–5.8. However, the phytoplankton community assemblage and abundance still resembles one of an acidic environment. Species dominant in the lake prior to acidification have not successfully colonized the lake in spite of ample seed populations.

We have shown that the early recovery process for the phytoplankton community is not the exact inverse of acidification. Based on the results of Hultberg and Andersson (1982) and Scheider et al. (1976), we expect that full recovery will occur when pH is allowed to increase to a circumneutral level for 2–7 yr. This will cause acidophilic species to decline in abundance to a level sufficient for circumneutral species to reestablish their dominance.

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References

ALMER, B., W. DICKSON, C. EKSTRÖM, AND E. HÖRNSTRÖM. 1978. Sulfur pollution and the aquatic ecosystem, p. 271–311. *In* J. Nriagu [ed.] Sulfur in the environment. Part 2. John Wiley and Sons, New York, NY.

ARMSTRONG, F. A. J., AND D. W. SCHINDLER. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 171–187.

CLEUGH, T. R., AND B. W. HAUSER. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 129–137.

CRUIKSHANK, D. R. 1984. Whole lake chemical additions in the Experimental Lakes Area, 1969–1983. Can. Data Rep. Fish. Aquat. Sci. 449: iv + 23 p.

1986. Whole lake chemical additions in the Experimental Lakes Area, 1984–1986. Can. Data Rep. Fish. Aquat. Sci. 580: iv + 10 p.

DIXIT, S. S., A. S. DIXIT, AND J. P. SMOL. 1989. Lake acidification recovery can be monitored using chrysophycean microfossils. Can. J. Fish. Aquat. Sci. 46: 1309–1312.

FINDLAY, D. L. 1984. Effects on phytoplankton biomass, succession, and composition in Lake 223 as a result of lowering pH levels from 5.6 to 5.2 data from 1980 to 1982. Can. MS Rep. Fish. Aquat. Sci. 1761: iv + 10 p.

FINDLAY, D. L., AND S. E. M. KASIAN. 1986. Phytoplankton responses to acidification of Lake 223, Experimental Lakes Area, northwestern Ontario. Water Air Soil Pollut. 30: 719–726.

1987. Phytoplankton community responses to nutrient additions in Lake 226, Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 35–46.

1990. Freshwater phytoplankton communities of lakes experimentally acidified with sulfuric and nitric acids. Can. J. Fish. Aquat. Sci. 47: 1378–1386.

HEALEY, F. P. 1973. Characteristics of phosphorus deficiency in *Anabaena*. J. Phycol. 9: 383–394.

1975. Physiological indicators of nutrient deficiency in algae. Fish. Mar. Serv. Res. Dev. Tech. Rep. 585: 30 p.

HULTBERG, H., AND I. B. ANDERSSON. 1982. Liming of acidified lakes: induced long-term changes. Water Air Soil Pollut. 18: 311–331.

KWIATKOWSKI, R. E., AND J. C. ROFF. 1976. Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Can. J. Bot. 54: 2546–2561.

NAUWERCK, A. 1963. Die beziehungen zwischen zooplankton und phytoplankton in see Erken. Symb. Bot. Upsal. 17: 163 p.

PROKOPOWICH, J. 1979. Chemical characterization of epilimnetic waters of unenriched lakes in the Experimental Lakes Area, northwestern Ontario. Can. Fish. Mar. Serv. Tech. Rep. 873: iv + 41 p.

SAS INSTITUTE INC. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute Inc., Cary, NC. 956 p.

SCHEIDER, W. A., B. CAVE, AND J. JONES. 1976. Reclamation of acidified lakes near Sudbury, Ontario, by neutralization and fertilization. Ontario Ministry of the Environment, Toronto, Ont. 56 p.

SCHINDLER, D. W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science (Wash., DC) 184: 879–899.

SCHINDLER, D. W., K. H. MILLS, D. F. MALLEY, D. L. FINDLAY, J. A. SHEARER, I. J. DAVIES, M. A. TURNER, G. A. LINDSEY, AND D. R. CRUIKSHANK. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

SCHINDLER, D. W., R. WAGEMANN, R. B. COOK, T. RUSZCZYNSKI, AND J. PROKOPOWICH. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37: 342–354.

SHEARER, J. A. 1978. Two devices for obtaining water samples integrated over depth. Can. Fish. Mar. Serv. Tech. Rep. 772: iv + 9 p.

VOLLENWEIDER, R. A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Tech. Rep. O.E.C.D. Paris Das/CSI/68.27: 1–182.

WASHINGTON, H. G. 1984. Review: diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. Water Res. 186: 653–694.

WATERS, T. F. 1956. The effects of lime application to acid bog lakes in northern Michigan. Trans. Am. Fish. Soc. 86: 329–344.

YAN, N. E., AND P. J. DILLON. 1984. Experimental neutralization of lakes near Sudbury, Ontario, p. 417–456. *In* J. R. Nriagu [ed.] Environmental impact of smelters. Vol. 15. John Wiley and Sons, New York, NY.

YAN, N. E., AND P. STOKES. 1978. Phytoplankton of an acidic lake, and its responses to experimental alteration of pH. Environ. Conserv. 5: 93–100.

Effects of Whole-Lake Base Addition on the Optical Properties of Three Clearwater Acidic Lakes

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Data describing changes in the attenuation, absorption, and scattering of photosynthetically active radiation (PAR) were collected over a 5-yr period from three limed lakes and an untreated reference site. Light attenuation rose significantly after liming. Short-term changes in light attenuation were attributed to increased scattering by suspended calcite particles; such changes persisted for fewer than 15 d due to the rapid dissolution of the calcite. Long-term increases in light attenuation corresponded to increases in light absorption, which were attributed to higher concentrations of light-absorbing substances (gelbstoff) as indicated by higher lake dissolved organic carbon (DOC) concentrations. Changes in chlorophyll did not play an important role in regulating light attenuation except during the first summer following liming. Reacidification of one of the three limed lakes was accompanied by a decrease in lake DOC concentrations, followed by a gradual decrease in light attenuation and absorption. Within 3 yr after liming, the chemical and optical characteristics of this site were comparable with the pretreatment period. Results from lake liming experiments are consistent with the hypothesis that changes in the photochemical properties and/or the concentration of light-absorbing organic compounds are important mechanisms by which acidification alters the optical properties of lakes.

Pendant 5 ans, on a recueilli des données décrivant l'atténuation, l'absorption et la diffusion du rayonnement photosynthétiquement actif (RPA) dans trois lacs chaulés et un site témoin non traité. Le taux d'atténuation de la lumière a nettement augmenté après le chaulage. Les variations à court terme du taux d'atténuation de la lumière sont attribuées à une plus forte diffusion par les particules de calcite en suspension. Ces variations n'ont pas duré plus de 15 jours à cause de la solution rapide du calcite. Des augmentations à long terme du taux d'atténuation de la lumière correspondent à des augmentations du niveau d'absorption de la lumière attribuées à de plus fortes concentrations de substances photoabsorbantes (gelbstoff) comme le révèlent les concentrations plus élevées de carbone organique dissous (COD). Les variations de la teneur en chlorophylle n'ont pas jouer un rôle important dans la modulation du taux d'atténuation de la lumière, sauf pendant le premier été après le chaulage. La réacidification d'un des trois lacs chaulés a été accompagnée d'une baisse des concentrations de COD suivie d'une baisse graduelle du taux d'atténuation et d'absorption de la lumière. Trois ans après le chaulage, les caractéristiques optiques et chimiques de ce site étaient analogues à celles observées avant le chaulage. Les résultats d'expériences sur le chaulage de lacs sont compatibles avec l'hypothèse que les variations des propriétés photochimiques et/ou de la concentration de composés organiques photoabsorbantes sont d'importants mécanismes par l'entremise desquels l'acidification modifie les propriétés optiques des eaux lacustres.

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Atmospheric deposition of strong acids has been accompanied by changes in the chemical and biotic characteristics of aquatic ecosystems in North America and Europe. Biotic responses are attributed to changes in lake chemistry, particularly elevated concentrations of aluminum (Driscoll et al. 1980). Biotic processes may also be affected by changes in the thermal and optical characteristics of acidified lakes. Almer et al. (1974) reported that the transparency of Lake Stora Skarsjon in Sweden increased as the lake acidified between 1943 and 1973. Similarly, Schofield (1972) reported that decreases in pH were accompanied by an increase of mean transparency for 14 Adirondack lakes sampled during 1950–53 and in 1967. Yan (1983) described changes in transparency following treatment of an acidified lake with base materials. Secchi depth was reduced during the period when lake pH was maintained above 6.0 but returned to pretreatment values as the lake reacidified. Light attenuation in the epilimnion of Lake 223 (Experimental Lakes Area (ELA), Ontario) decreased as the lake was experimentally acidified (Shearer et al. 1987).

Kramer (1978) suggested that increased transparency in acidified lakes was attributable to reductions in phytoplankton standing crop. However, changes in phytoplankton abundance have not been consistently correlated with acidification (Baker 1984). On the one hand, studies on lakes in Sudbury, Ontario

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TABLE 1. Morphometric and chemical characteristics of the four study sites. Chemical data are approximate ranges observed prior to treatment during summer stratification.

Site characteristics	Cranberry Pond	Wood Lake	Dart's Lake	Little Simon Pond
Lake area ($m^2 \times 1000$)	72	230	570	626
Lake volume ($m^3 \times 1000$)	207	802	4150	6340
Maximum depth (m)	7	12	15	33
Mean depth (m)	2.9	3.5	7.3	10.1
Residence time (d)	90	214	80	450
pH	4.7 – 4.9	4.9 – 5.2	5.0 – 5.5	5.4 – 5.7
ANC (μeq/L)	−25 – −5	−15 – +5	−10 – +10	−10 – +10
Monomeric Al (μmol/L)	5 – 20	2 – 12	5 – 10	0.5 – 3.5
DOC (μmol C/L)	250 – 450	150 – 300	240 – 400	200 – 300

TABLE 2. Analytical procedures and sampling frequency for physical and chemical data collected at the study sites (Intg = integrated water column sample).

Parameter	Method	Sampling frequency	Depth intervals
Temperature	YSI S-C-T meter	5–10 d	0.5–1 m
Light	Protomatic photometer	5–10 d	0.5–1 m
pH	Potentiometric	5–10 d	Intg
Acid-neutralizing capacity (ANC)	Strong acid titration, Gran plot analyses	5–10 d	Intg
Chlorophyll	Acetone extraction, spectrophotometric analyses	5–10 d	2 m
Dissolved organic carbon (DOC)	Persulfate digestion, IR detection of CO_2	3 wk	1–5 m
Dissolved oxygen	Winkler titration	1–3 wk	1–5 m
Fe	Atomic absorption spectrophotometry	3 wk	1–5 m
Turbidity	Nephelometric	3 wk	1–5 m

(Kwiatkowski and Roff 1976), the Adirondacks (Hendrey 1980), and Florida (Crisman et al. 1980) reported that the primary productivity of acidic lakes is lower relative to circumneutral waters in the same region. On the other hand, surveys in Norway (Raddum et al. 1980) and Sweden (Almer et al. 1978) indicated that primary productivity was greater than or equal to that of circumneutral lakes. Phytoplankton abundance in Lake 223 did not decline during experimental acidification; however, treatment of this site was not accompanied by an increase in aluminum comparable with concentrations measured in acidified lakes occurring in Sweden and the northeastern United States (Schindler et al. 1985). Binding and removal of orthophosphate by aluminum has been proposed as a mechanism accounting for oligotrophication of lakes during acidification (Grahn et al. 1974).

A number of investigators have documented the correlation between transparency and phytoplankton biomass for lakes exhibiting a range of trophic conditions (e.g. Bindloss 1976; Jewson 1977; Carlson 1977). However, lakes occurring in acid-sensitive regions are typically dilute and oligotrophic. Since phytoplankton abundance is low, other factors may exert a greater influence on light attenuation. Kirk (1976) reported that gelbstoff was the dominant component attenuating light in the inland waters of Australia. "Gelbstoff" or "gilvin" is the general term applied to a diverse array of chemical compounds which constitute dissolved yellow pigments in natural waters. Almer et al. (1978) proposed that coagulation and removal of dissolved yellow substances by aluminum may account for increased clarity in acidic lakes. Data collected by Effler et al. (1985) supported this hypothesis. They reported that seasonal patterns of light attenuation in an acidic Adirondack lake (Dart's Lake) corresponded to changes in dissolved organic carbon (DOC) that were coupled with the loss of aluminum from the water column. Phytoplankton did not play an important role in regulating light attenuation at this site.

In this study, the effects of whole-lake base addition on the optical properties of three acidic Adirondack lakes were examined. Data describing changes in the attenuation, absorption, and scattering of photosynthetically active radiation (PAR) were collected at three treated sites and at a reference site. DOC was measured to indicate changes in the concentration of light-absorbing organic substances (gelbstoff). Chlorophyll and DOC concentrations were monitored at all sites to interpret their role in regulating light attenuation.

Treatment Design and Site Description

Study sites are located in predominantly forested and undeveloped watersheds in the west-central (Cranberry Pond, Woods Lake, and Dart's Lake) and central (Little Simon Pond)

TABLE 3. Symbols and method of calculating various irradiation variables.

Variable name	Symbol	Equation
Downwelling irradiance	E_d	
Upwelling irradiance	E_u	
Vector irradiance	E	$E = E_d - E_u$
Scalar irradiance	E_o	$E_o = E_d + E_u$
Average cosine	$\bar{\mu}$	$\bar{\mu} = E/E_o$
Attenuation coefficient for downwelling irradiance	K_d	K_d = slope of $\ln(E_d)$ vs. depth
Attenuation coefficient for net irradiance	K_e	K_e = slope of $\ln(E)$ vs. depth
Absorption coefficient	a	$a = K_e \cdot \bar{\mu}$
Scattering coefficient	b	$b = (K_d^2 - a^2)/0.25a$

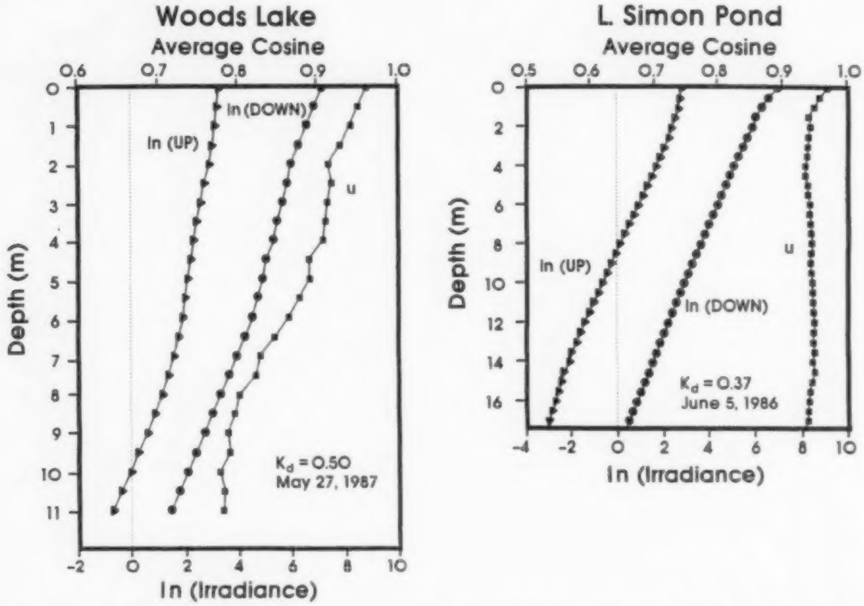

FIG. 1. Depth profiles of upwelling and downwelling irradiance and average cosine (μ) for Woods Lake and Little Simon Pond.

Adirondack Mountain region of New York State. Drainage lakes occurring in this region are typically dilute and oligotrophic and can be characterized as clearwater lakes on the basis of having low light attenuation coefficient ($K_d < 1.00$; P. Bukaveckas, unpubl. data).

Detailed descriptions of watershed hydrology and geology for the four study sites are presented in Staubitz and Zarriello (1989). Research at these sites is part of a multidisciplinary study of lake liming by investigators participating in the Lake Acidification Mitigation Project (LAMP). The purpose of this project is to document changes in the chemical and biological characteristics of clearwater acidic lakes following whole-lake base addition. An overview of the design and objectives of LAMP is presented in Porcella (1989).

Prior to base treatment, the chemical characteristics of the study sites were typical of clearwater acidic lakes occurring in the Adirondack region (Driscoll and Newton 1985). At all sites, sulfate was the dominant anion and the equivalence of acidic anions exceeded basic cations, resulting in low pH, reduced acid-neutralizing capacity (ANC), and generally high concentrations of aqueous aluminum (Table 1). All lakes and most inlet streams exhibited low concentrations of DOC, although elevated concentrations were measured in one of the inlets to Little Simon Pond. Detailed descriptions of lake chemistry are presented in Driscoll et al. (1987), Fordham and Driscoll (1989), and McAvoy and Driscoll (1989).

Cranberry Pond and Woods Lake were selected as sites typical of highly acidic Adirondack lakes. Both sites are small drainage lakes (<25 ha) that generally exhibited pH < 5 and ANC < 0 during the pretreatment period. The lakes occur within adjacent watersheds and therefore experience similar meteorological conditions. The lakes differ, however, in their hydraulic retention time (Table 1). Woods Lake has approximately 4 times the volume of Cranberry Pond and therefore exhibits a longer hydraulic retention time. Calcium carbonate added to Cranberry Pond was rapidly depleted and the lake reacidified within 1 yr after treatment. Data collected following reacidification provided an opportunity to examine changes in optical characteristics associated with increasing acidity. Woods Lake was retreated to maintain a circumneutral pH over a 4-yr period.

Little Simon Pond was selected as a site typical of larger, moderately acidic lakes in the Adirondack region. This site contained higher concentrations of basic cations relative to Cran-

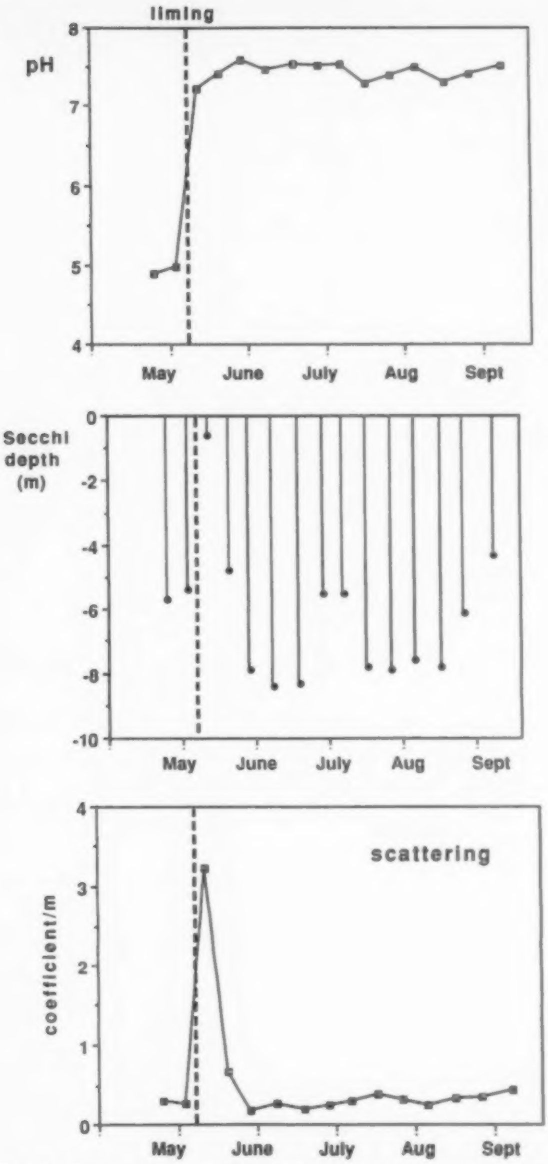

FIG. 2. Changes in pH, Secchi depth, and light scattering following treatment of Woods Lake on May 30–31, 1985.

berry Pond and Woods Lake and, as a result, had higher pH and ANC (pH = 5.5, ANC > 0) and lower aluminum concentrations (Table 1). Little Simon Pond had a longer hydraulic residence time than the two smaller lakes and therefore did not experience significant reacidification during the posttreatment period.

The three experimental sites were treated by application of a highly pure calcite slurry (98% calcium carbonate). The slurry was applied by helicopter on a volumetric basis to achieve a uniform target pH/ANC. Treatments were conducted in May 1985 at Cranberry Pond (7 tonnes (t) dry weight $CaCO_3$) and Woods Lake (23 t) and in August 1986 at Little Simon Pond (86 t). Woods Lake was retreated (35 t) during fall 1986. An account of the treatment program is available in Porcella (1987). Treatment effects on lake chemistry are described in Fordham and Driscoll (1989) and Driscoll et al. (1989).

Dart's Lake was selected as the reference site because of its proximity to the experimental lakes. It exhibited chemical and morphometric features within the range of the three treated lakes. In addition, extensive background data were available for Dart's Lake, including a long-term record of lake chemistry (Driscoll and Schafran 1984; Schafran and Driscoll 1987) and a previous study of optical properties (Effler et al. 1985).

Sampling and Analytical Methods

Irradiance profiles, temperature profiles, and lake water samples were collected at a single station over the deepest portion of each lake basin. Sampling frequency and analytical procedures for chemical parameters are summarized in Table 2. Irradiance was measured with a photometer equipped with upward and downward spherical sensors exposed as hemispheres. The wavelength response of this meter is approximately 300–770 nm and is similar to the ideal quantum response for PAR (Field and Effler 1983). Light data were collected during June–August 1984 and May–September 1985–88. On each sampling date, two to four replicate profiles were recorded between 11:00 and 13:00.

Depth profiles of downwelling (E_{od}) and upwelling (E_{ou}) irradiance were analyzed using the methods of Kirk (1983) to estimate coefficients of light attenuation (K_d), absorption (a), and scattering (b). The conformations of the upward and downward sensors were appropriate for measuring quantities proportional to the sum of scalar and vector irradiance (Hojerslev 1975). Vector irradiance (E) was calculated as downwelling minus upwelling radiation; scalar irradiance (E_o) was calculated as the sum of upwelling and downwelling radiation (symbols and calculations are summarized in Table 3).

Coefficients of light attenuation were calculated as the slope of a line obtained by plotting the natural logarithm of irradiance against depth. The attenuation coefficient for downwelling irradiance (K_d) was determined from a linear regression of the natural logarithm of downwelling irradiance against depth. The attenuation coefficient for net downward irradiance (K_e) was estimated from a linear regression of the natural logarithm of downwelling minus upwelling irradiance against depth. Correlation coefficients derived from fitting linear regressions to irradiance data were uniformly high ($r > 0.98$). The standard error of the slope was less than 5% for approximately 90% of the regressions.

The absorption coefficient (a) was calculated as the product of K_e and the average cosine (μ). The average cosine represents the ratio of vector ($E_d - E_u$) to scalar ($E_d + E_u$) irradiance. This value varies as a function of the amount of upwelling radiation within the water column. Since scattering of light increases upwelling radiation, an increase in the relative contribution of scattering causes a decrease in estimates of the average cosine. The scattering coefficient (b) was estimated from K_d and a according to the expression developed by Kirk (1983) (given in Table 3). The method by which the scattering coefficient is calculated assumes that upwelling irradiance originates entirely from the scattering of light within the water column. Light reflecting from the bottom will increase upwelling irradiance and result in overestimation of the scattering coefficient. To assess the validity of this assumption, estimates of average cosine were plotted against depth for each sampling date at all sites.

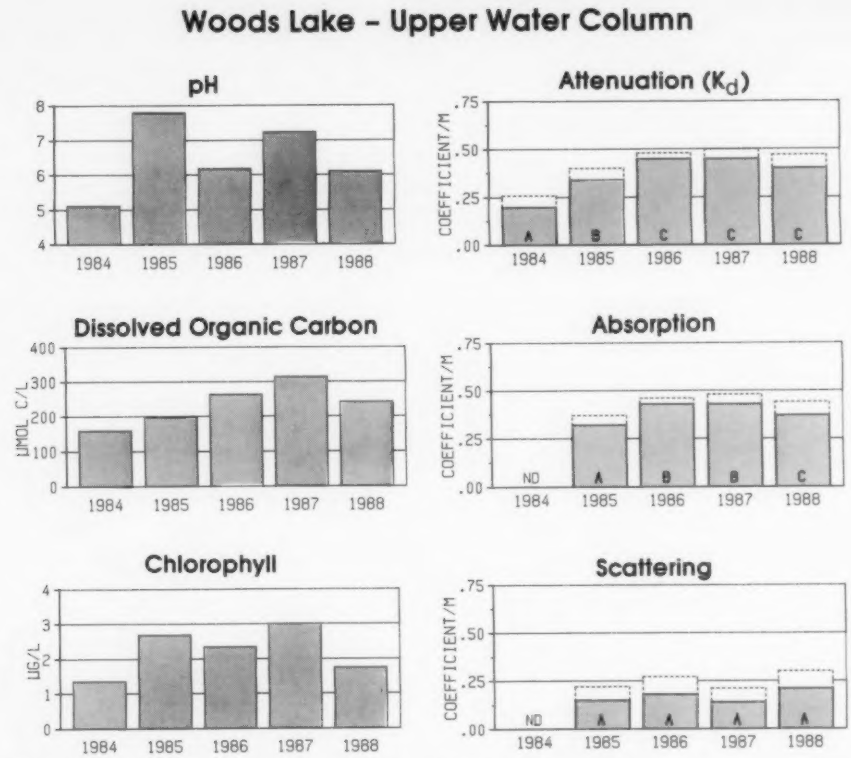

FIG. 3. Averages of summer pH, light coefficients, DOC, and chlorophyll for the upper water column of Woods Lake (treated May 1985 and September 1986). Broken lines indicate 1 standard deviation above the mean. Bars denoted by the same letter are not significantly different.

Reflectance from the bottom did not contribute significantly to measurements of upwelling irradiance. The average cosine exhibited little variability with depth for profiles collected at Little Simon Pond (Fig. 1), Cranberry Pond, and Dart's Lake. Estimates of average cosine calculated for the middle of the photic zone (10% light level) were representative of water column scattering conditions and were used to calculate absorption coefficients at these sites. At Woods Lake, however, profiles of average cosine obtained during the posttreatment period exhibited significant variability with depth (Fig. 1). Therefore, separate attenuation, absorption, and scattering coefficients were calculated for the upper (0–6 m) and lower (6–12 m) portions of the water column to adequately characterize the optical properties of this site. Light coefficients for the upper and lower water column were calculated using the mean μ for each zone.

To further define changes in attenuation with depth, coefficients were calculated for specific layers within the water column. For example, an attenuation coefficient for 7 m was calculated from irradiance measurements recorded at 6, 7, and 8 m. Since these regressions were based on only three data points, correlation coefficients were lower than those for regressions of whole water column data. For layers occurring in the middle or lower water column, the standard error of the slope was less than 10% for approximately 95% of the calculated coefficients. Near the lake surface, wave action resulted in higher variance and therefore greater error for slope estimates. Scattering and absorption coefficients for specific depths were calculated using the K_d for that layer and a mean value of the average cosine for the three depths.

Statistical analyses were performed using the Statistical Analyses System (SAS). Between-year comparisons at each site were based on one-way analyses of variance using a repeated measures Generalized Linear Model design. Student–Newman–Keuls tests were used to evaluate the hypothesis that coefficients were significantly different during the pretreatment period. Coefficients derived from upper and lower water column data in Woods Lake were analyzed by paired observation t-tests. Unless otherwise noted, statistical significance refers to the $p < 0.05$ level.

Results

Short-term effects of base addition on light attenuation were similar at the three treated sites. Data collected at Woods Lake are used to illustrate short-term changes. Application of the calcite slurry resulted in an immediate reduction in lake transparency as indicated by the decrease in Secchi depth from 6 m to less than 1 m (Fig. 2). Corresponding to the decrease in Secchi depth, K_d increased from 0.41 to 0.74 within 3 d following base addition. The increase in K_d is attributed to greater light scattering due to the presence of calcite particles suspended in the water column. Estimates of scattering were 10-fold higher immediately after liming (Fig. 2). Changes in light attenuation associated with increased scattering persisted for fewer than 15 d due to the rapid dissolution of the calcite particles. Data collected during the 15 d period immediately following treatment were omitted from statistical analyses of long-term trends to exclude the transient effects of the liming.

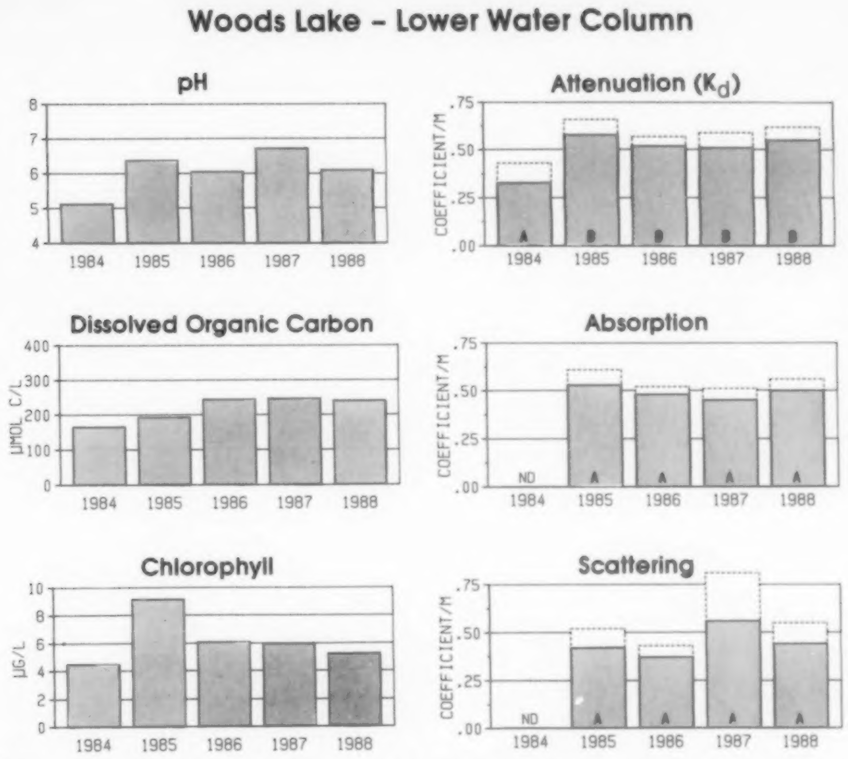

FIG. 4. Averages of summer pH, light coefficients, DOC, and chlorophyll for the lower water column of Woods Lake. Broken lines indicate 1 standard deviation above the mean. Bars denoted by the same letter are not significantly different.

Light attenuation in both the upper (0–6 m) and lower (6–12 m) water columns of Woods Lake was significantly higher throughout the posttreatment period relative to preliming data. In the upper water column (Fig. 3), mean K_d increased during the first summer after liming (from 0.20 to 0.34) and during the second summer after liming (from 0.34 to 0.45). The increase in attenuation observed during 1986 corresponded to an increase in light absorption and was attributed to the increase in mean DOC concentrations (from 198 to 264 µmol C/L). Estimates of scattering were not significantly different between years. In the lower water column (Fig. 4), mean K_d increased from 0.33 in 1984 to 0.54 (average of means for 1985–88). Increases in light attenuation coincided with increases in both DOC and chlorophyll concentrations in the lower water column. Mean DOC concentrations increased from 165 µmol C/L in 1984 to over 240 µmol C/L during 1986–88. Chlorophyll increased from 4.47 µg/L (summer mean prior to liming) to 9.14 µg/L in 1985.

Comparisons of upper and lower water column data indicated that light attenuation was consistently greater in the lower water column of Woods Lake. Increased attenuation in the deeper layer is attributed to differences in light scattering, since absorption coefficients were not significantly different between the upper and lower water columns except in 1985. Scattering coefficients calculated for the lower water column were 2–3 times higher compared with those for the upper water column.

Differences in scattering between the upper and lower water columns may have been due to differences in phytoplankton abundance. Chlorophyll concentrations were 2–3 times higher in the lower water column relative to the upper column. Analyses of seasonal trends suggested that other mechanisms may have also been important in regulating scattering. The highest mean and range of scattering coefficients were observed in the lower water column in 1987 (Fig. 4) when the water column failed to mix completely in the spring. Highest measurements of scattering and turbidity were recorded during May and July and were twofold higher relative to data collected in June and August (Fig. 5). The July increase in scattering coincided with the onset of anoxic conditions in the hypolimnion. Anoxia promotes the release of ferrous iron from the sediments which may be reoxidized when exposed to oxygen in the water column (Wetzel 1983). The presence of iron – organic carbon complexes or iron hydrolysis products in the hypolimnion may have resulted in greater light scattering. However, chemistry data collected during this period indicated that iron concentrations were highest during the period following the July increase in scattering (Fig. 5). Similarly, it was unlikely that phytoplankton were a causative factor, since the increase in scattering also preceded the late-summer chlorophyll peak. Increased scattering may have been due to an increase in hypolimnetic bacterial densities. Since data describing changes in bacterial abundance were not collected as part of this study, we analyzed changes in the rate of hypolimnetic oxygen depletion as an indicator of changes in metabolic activity. The highest rate of oxygen depletion occurred between July 3 and 13 and coincided with the increase in scattering. During this period, the mass of oxygen in the hypolimnion was reduced by 37%.

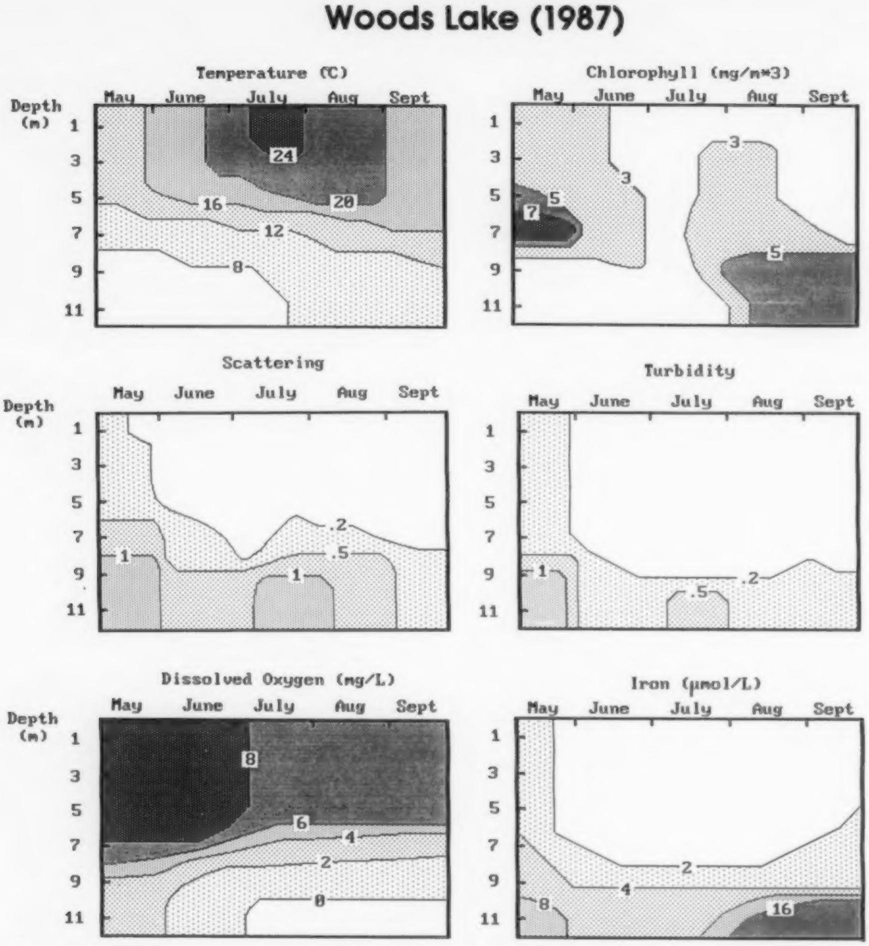

FIG. 5. Isopleths of scattering, turbidity, dissolved oxygen, iron, chlorophyll, and temperature for Woods Lake in 1987.

At Little Simon Pond, light attenuation also increased after base addition (Fig. 6). Light attenuation was significantly higher during 1987 and 1988 after liming in the fall of 1986. Increases in K_d corresponded to increases in light absorption and DOC concentrations. No increase in chlorophyll was observed after liming, and estimates of scattering were not significantly different between years.

The effects of base addition on light attenuation in Cranberry Pond were similar to those observed in Woods Lake. Light attenuation was significantly higher after liming relative to the preliming year (Fig. 7). The increase in light attenuation coincided with increases in both DOC and chlorophyll concentrations, but persisted longer. Treatment effects on lake chemistry persisted for less than 1 yr, since added calcium carbonate was rapidly flushed from the lake. By 1986, the pH of Cranberry Pond was similar to pretreatment conditions. Reacidification was accompanied by a reduction in DOC and chlorophyll concentrations which was followed by a gradual decrease in light attenuation. By 1988, light attenuation was not significantly different from the pretreatment period. Decreases in K_d following reacidification were attributed to reductions in both light absorption and scattering. The highest absorption and scattering coefficients were measured in 1985 when pH, chlorophyll, and DOC were also highest.

No changes in pH or light attenuation were observed at the reference site during the 5-yr study. Summer means of attenuation, scattering, and absorption coefficients were not significantly different between years (Fig. 8). The optical properties of the reference lake were similar to those of the three experimental lakes. Summer means and seasonal ranges of the three light coefficients (K_d, a, and b) were comparable with those calculated for the upper water column of Woods Lake (Fig. 3), Little Simon Pond (Fig. 6), and Cranberry Pond (Fig. 7). At all sites, estimates of scattering were low relative to absorption coefficients (ratio of $b/a < 0.5$), suggesting that absorption was the dominant mechanism attenuating light. However, scattering was an important component of light attenuation of the lower water column of Woods Lake (ratio of $b/a \approx 1.0$; Fig. 4).

Discussion

Neutralization of lake acidity by base addition resulted in both short-term and long-term increases in light attenuation at all sites. Short-term changes were due to increases in light scat-

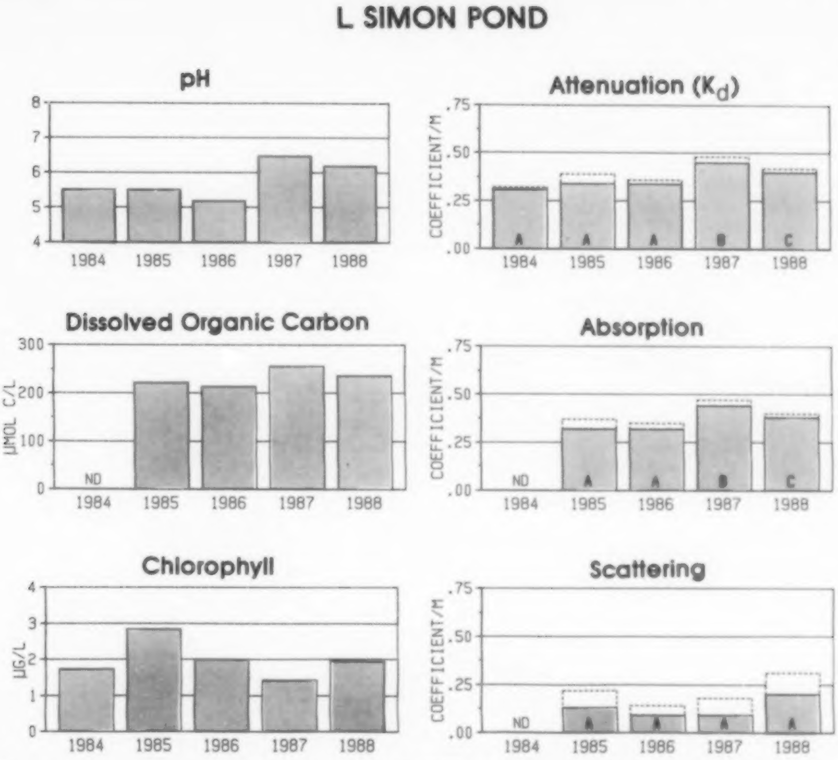

FIG. 6. Averages of summer pH, light coefficients, DOC, and chlorophyll for Little Simon Pond (limed August 1986). Broken lines indicate 1 standard deviation above the mean. Bars denoted by the same letter are not significantly different.

tering and were attributed to calcite particles suspended in the water column. Long-term changes were associated with increases in light absorption which corresponded to increases in lake DOC concentrations. No change in K_d was observed at the reference site during the period of study.

Increases in attenuation associated with liming represent a significant reduction in irradiance flux to deeper layers. In Woods Lake, the availability of PAR at the lower boundary of macrophyte growth (5 m) decreased from 38% of subsurface irradiance to less than 10% by the second summer after treatment. Reductions in irradiance levels were accompanied by declines in macrophyte growth (Bukaveckas 1988a). At Little Simon Pond, the increase in attenuation after liming (from 0.34 in 1986 to 0.45 in 1987) corresponded to a 50% decrease in light flux to the metalimnion. Reductions in light penetration may have accounted for the absence of the deepwater chlorophyll maximum observed in previous years (Bukaveckas 1988b).

Changes in light attenuation were expressed per unit change in pH to facilitate between-site comparisons (Table 4). Woods Lake and Little Simon Pond exhibited a 0.11 increase in mean summer K_d per unit increase in pH. A 0.10 increase in K_d per unit increase in pH was observed following the liming of Cranberry Pond. A 0.10 increase in K_d per unit increase in pH was also observed following the liming of Lake Madeleine (P. Bukaveckas, unpubl. data). Lake Madeleine lies in an adjacent watershed to Little Simon Pond and was treated concurrently. Prior to base addition, Lake Madeleine exhibited a slightly greater ANC (0 to +20 µeq/L) and higher pH (pH > 6) relative to Little Simon Pond. The reacidification of Cranberry Pond resulted in a 0.09 decrease in K_d per unit decrease in pH. Similarly, a 0.10 decrease in K_d per unit pH was observed during the experimental acidification of Lake 223. At sites for which attenuation data were unavailable, the change in Secchi depth per unit change in pH was calculated. A unit change in lake pH resulted in a 1- to 3-m change in Secchi depth. The effects of liming on K_d and Secchi depth were similar for lakes that differed in pretreatment pH and geographic location. Reacidification of limed lakes and the experimental acidification of Lake 223 also resulted in a similar change in K_d. These analyses suggest that a uniform change in transparency per unit change in pH is observed in clearwater lakes. Liming of bog ponds causes an increase in transparency as humic materials are removed from the water column (Hasler et al. 1951; Kitchell and Kitchell 1980); therefore, colored lakes were not included in these comparisons.

The consistency of these effects suggests that there may be a common mechanism by which changes in lake acidity regulate light attenuation. Kramer (1978) suggested that higher transparency in acidic lakes was due to lower phytoplankton abundance. A number of investigators have reported increases in phytoplankton biomass following base addition (e.g. Eriksson et al. 1983; Broberg 1987), although in some studies no change was observed (Yan and Dillon 1984; Brettum and Hindar 1985). Average summer chlorophyll concentrations increased twofold following the liming of Cranberry Pond and Woods Lake. In Woods Lake, seasonal changes in scattering were correlated with chlorophyll concentrations (r = 0.77)

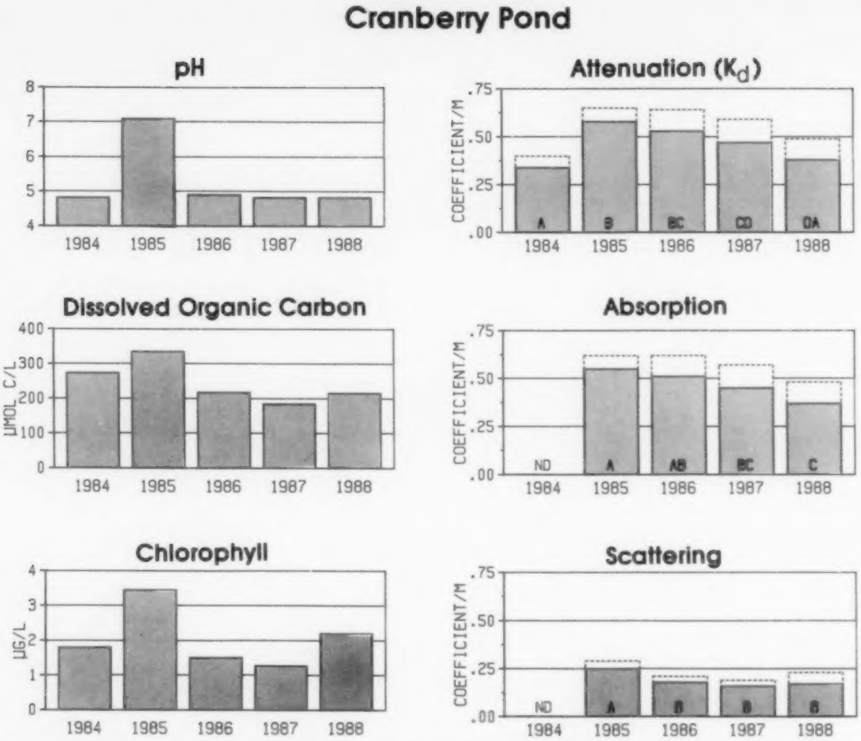

FIG. 7. Averages of summer pH, light coefficients, DOC, and chlorophyll for Cranberry Pond (treated May 1985). Broken lines indicate 1 standard deviation above the mean. Bars denoted by the same letter are not significantly different.

TABLE 4. Changes in pH, light attenuation, and Secchi depth during liming and acidification studies.

Site	Treatment	pH		K_d (m^{-1})		Secchi (m)				
		Pre-	Post-	Pre-	Post-	Pre-	Post-	K_d /pH	Secchi/pH	Source[a]
Cranberry Pond	Liming	4.82	7.09	0.34	0.58	—	—	0.10	—	1
	Reacid	7.09	4.83	0.58	0.38	—	—	0.09	—	
Woods Lake	Liming	5.10	6.96	0.23	0.43	8.8	7.0	0.11	1.0	1
Little Simon Pond	Liming	5.19	6.47	0.34	0.45	9.0	7.8	0.11	0.9	1
Lake Madeleine (Adirondacks)	Liming	6.26	6.63	0.23	0.27	—	—	0.11	—	2
Lohi Lake (Sudbury, Ont.)	Liming	4.39	6.04	—	—	8.8	5.6	—	1.9	3
	Reacid	6.09	4.74	—	—	5.7	7.6	—	1.4	
Middle Lake	Liming	4.40	7.01	—	—	10.5	2.9	—	2.9	3
Lake 223 (ELA, Ontario)	Exp. acid	6.68	5.11	0.42	0.26	—	—	0.10	—	4
S. Holmevatten (Sweden)	Liming	4.50	7.50	—	—	14.0	7.0	—	2.3	5
Lake Gardsjon (Sweden)	Liming	4.65	7.45	—	—	10.0	5.2	—	1.7	6
Stora Skarsjon (Sweden)	Acid	6.25	4.50	—	—	5.0	12.0	—	4.0	7

[a] 1, This study; 2, P. Bukaveckas (unpubl. data); 3, Yan and Dillon (1982); 4, Shearer et al. (1987); 5, Hasselrot et al. (1984); 6, Henrikson et al. (1984); 7, Almer et al. (1974).

during the first summer after treatment (1985). However, short-term changes in phytoplankton abundance may represent an extreme response to the experimental manipulation that would not be indicative of phytoplankton responses to gradual changes in lake acidity. Chlorophyll concentrations during the first summer after treatment were considerably higher than those observed prior to liming or during subsequent summers (Bukaveckas 1988b, 1989). The dramatic increase in phytoplankton

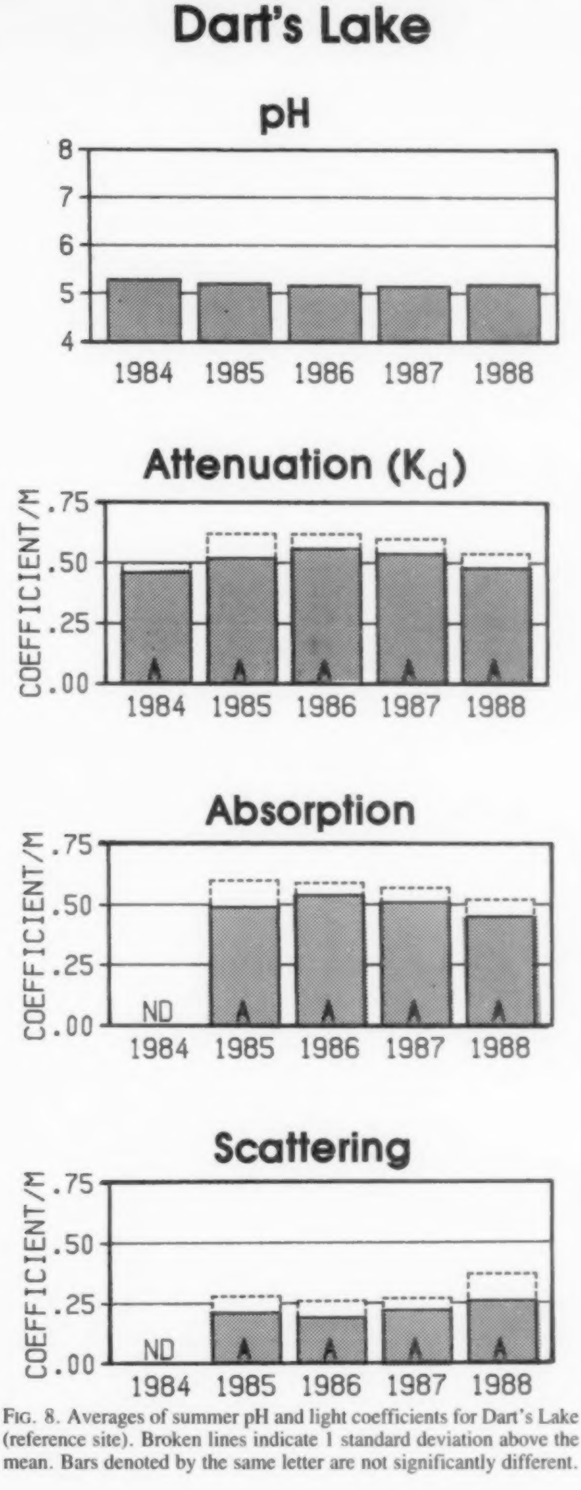

FIG. 8. Averages of summer pH and light coefficients for Dart's Lake (reference site). Broken lines indicate 1 standard deviation above the mean. Bars denoted by the same letter are not significantly different.

abundance that occurred 2 mo after treatment is attributed to a release from grazing pressure, since base addition resulted in 90% mortality of the zooplankton community (Schaffner 1989).

Although increases in phytoplankton abundance contributed to greater scattering and higher attenuation during the first summer after liming, long-term changes in K_d were not correlated with changes in chlorophyll. In Cranberry Pond, light attenuation was significantly higher between 1985 and 1987 whereas chlorophyll concentrations were higher only during 1985. At Little Simon Pond, a significant increase in attenuation was observed during the first summer after liming, despite decreases in chlorophyll concentrations. Similarly, Yan (1983) reported that changes in Secchi depth following the liming of Lohi Lake were negatively correlated with pH, but were not correlated with chlorophyll concentrations or phytoplankton biomass.

Almer et al. (1978) proposed that the coagulation and removal of DOC by aluminum resulted in lower light absorption and therefore greater transparency in acidic lakes. Data collected in this study were consistent with their hypothesis. Long-term changes in light attenuation following liming corresponded to changes in light absorption. At Woods Lake and Little Simon Pond, posttreatment changes in light absorption were correlated with changes in DOC. Increases in DOC during the posttreatment period may have been due to removal of aluminum from the water column. At Woods Lake, concentrations of inorganic (labile) monomeric aluminum decreased from 10 to less than 3μmol/L within 6–8 wk after liming (Driscoll et al. 1989). Increases in phytoplankton abundance may have also contributed to higher DOC concentrations through cellular excretion and lysis of senescing cells. Although phytoplankton production was higher during the posttreatment period, scattering was generally not an important component of light attenuation except in the lower water column of Woods Lake.

Reductions in light penetration at Woods Lake were positively correlated with changes in thermocline depth and epilimnetic thickness (Bukaveckas and Driscoll 1991). Liming resulted in a shift from weak to strong thermal stratification. Development of the hypolimnetic zone allowed biogeochemical processes to have a greater influence on chemical conditions in deeper layers. Greater hypolimnetic oxygen depletion was observed during the posttreatment period and was attributed to stronger thermal stratification, coupled with increased phytoplankton production. Development of anoxic conditions may have been an important factor influencing the optical properties of the lower water column in Woods Lake. Anoxia promotes the development of limnetic anaerobic bacterial communities and the release of ferrous iron and/or iron – organic carbon complexes from sediments. Higher bacterial densities and the presence of iron colloids may have contributed to greater light scattering in the hypolimnion. Thus, stronger thermal stratification during the posttreatment period may have promoted greater optical stratification in Woods Lake.

At Cranberry Pond, changes in light attenuation following reacidification corresponded to changes in light absorption. However, lake DOC concentrations did not decline during the period when decreases in mean summer K_d were observed (1986–88). This may be attributed to the fact that measurements of DOC are an imperfect estimate of changes in gelbstoff (the light-absorbing component of DOC). Changes in light absorption during this period may have been due to changes in the light-absorbing properties of organic compounds which are not reflected in measurements of DOC concentration. This mechanism was also proposed to account for the decrease in K_d observed during the experimental acidification of Lake 223 (Shearer et al. 1987). To evaluate the relative importance of changes in the spectral properties of organic compounds, additional data are required describing their photochemical properties. Specifically, measurements of light absorption by naturally occurring dissolved organic compounds under a range of pH conditions are needed to assess their role in regulating light attenuation.

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References

Almer, B., W. Dickson, C. Ekstrom, and E. Hornstrom. 1978. Sulfur pollution and the aquatic ecosystem, p. 273–311. *In* J. Nriagu [ed.] Sulfur in the environment. Part II. John Wiley & Sons, New York, NY.

Almer, B., W. Dickson, C. Ekstrom, E. Hornstrom, and U. Miller. 1974. Effects of acidification on Swedish lakes. Ambio 3: 30–36.

Baker, J. P. 1984. Effects of acidification on phytoplankton, Chap. 5, p. 47–57. *In* A. P. Altshuller and R. A. Linthurst [ed.] The acidic deposition phenomenon and its effects. Critical Assessment Review Papers. Vol. II. EPA-600/8-830-016BF, Washington, DC.

Bindloss, M. E. 1976. The light climate of Loch Leven, a shallow Scottish lake, in relation to primary production by phytoplankton. Freshwater Biol. 6: 501–518.

Brettum, P., and H. Hindar. 1985. Effects of lime treatment on the biological system, p. 79–108. *In* K. Baalsrud, A. Hindar, M. Johannessen, and D. Matzow [ed.] Lime treatment against acid water. Norwegian Institute for Water Research, Oslo, Norway.

Broberg, O. 1987. Delayed nutrient responses to the liming of Lake Gardsjon, Sweden. Hydrobiologia 150: 22–27.

Bukaveckas, P. A. 1988a. Effects of calcite treatment on primary producers in acidified Adirondack lakes. I. Response of macrophyte communities. Lake Reserv. Manage. 4: 107–113.

1988b. Effects of lakes liming on phytoplankton growth in acidic Adirondack lakes. Water Air Soil Pollut. 41: 223–240.

1989. Effects of calcite treatment on primary producers in acidified Adirondack lakes. II. Short-term response by phytoplankton communities. Can. J. Fish. Aquat. Sci. 46: 352–359.

Bukaveckas, P. A. and C. T. Driscoll. 1991. Effects of whole-lake base addition on the thermal properties of three clearwater acidic lakes. Water Air Soil Pollut. (In press)

Carlson, R. E. 1977. A trophic state index for lakes. Limnol. Oceanogr. 22: 361–369.

Crisman, T. L., R. L. Schulze, P. L. Brezonik, and S. Bloom. 1980. Acid precipitation: the biotic response in Florida lakes, p. 296–297. *In* D. Drablos and A. Tollan [ed.] Ecological impact of acid precipitation. SNSF Project, Oslo, Norway.

Driscoll, C. T., W. A. Ayling, G. F. Fordham, and L. M. Oliver. 1989. Chemical response of lakes treated with $CaCO_3$ to reacidification. Can. J. Fish. Aquat. Sci. 46: 258–267.

Driscoll, C. T., J. P. Baker, J. J. Bisogni, and C. L. Schofield. 1980. Effects of aluminum speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

Driscoll, C. T., G. F. Fordham, W. A. Ayling, and L. H. Oliver. 1987. The chemical response of acidic lakes to calcium carbonate treatment. Lake Reserv. Manage. 3: 404–411.

Driscoll, C. T., and R. M. Newton. 1985. Chemical characteristics of acid-sensitive lakes in the Adirondack region of New York. Environ. Sci. Technol. 19: 1018–1124.

Driscoll, C. T., and G. C. Schafran. 1984. Short-term changes in the base neutralization capacity of an acidic Adirondack (NY) lake. Nature (Lond.) 310: 308–310.

Effler, S. W., G. C. Schafran, and C. T. Driscoll. 1985. Partitioning light attenuation in an acidic lake. Can. J. Fish. Aquat. Sci. 42: 1707–1711.

Eriksson, F., E. Hornstrom, P. Mossberg, and P. Nyberg. 1983. Ecological effects of lime treatment of acidified lakes and rivers in Sweden. Hydrobiologia 101: 145–164.

Field, S. D., and S. W. Effler. 1983. Light attenuation in Onondaga Lake, NY, USA. Arch. Hydrobiol. 98: 409–421.

Fordham, G. F., and C. T. Driscoll. 1989. Short-term changes in the acid/base chemistry of two acidic lakes following calcium carbonate treatment. Can. J. Fish. Aquat. Sci. 46: 306–314.

Grahn, O., H. Hultberg, and L. Landner. 1974. Oligotrophication — a self-accelerating process in lakes subjected to excessive supply of acid substance. Ambio 3: 93–94.

Hasler, A. D., O. M. Brynildson, and W. T. Helm. 1951. Improving conditions for fish in brown-water bog lakes by alkalization. J. Wildl. Manage. 15: 347–352.

Hasselrot, B., B. I. Andersson, and H. Hultberg. 1984. Ecosystem shifts and reintroduction of arctic char (*Salvelinus salvelinus*) after liming of a strongly acidified lake in Southwestern Sweden. Rep. Inst. Freshwater Res. Drottningholm 61: 78–92.

Hendrey, G. 1980. Effects of acidity on primary productivity in lakes: phytoplankton. *In* D. Shriner et al. [ed.] Atmospheric sulfur deposition — Environmental impact and health effects. Ann Arbor Science Publishers, Ann Arbor, MI.

Henrikson, L., H. G. Oscarson, and J. A. E. Stenson. 1984. Development of the crustacean zooplankton community after lime treatment of the fishless Lake Gardsjon, Sweden. Rep. Inst. Freshwater Res. Drottningholm 61: 104–114.

Hojerslev, N. 1975. A spectral light absorption meter for measurements in the sea. Limnol. Oceanogr. 20: 1024–1034.

Jewson, D. H. 1977. Light penetration in relation to phytoplankton content of the euphotic zone in Lough Neagh, N. Ireland. Oikos 28: 74–83.

Kirk, J. T. O. 1976. Yellow substance (gelbstoff) and its contribution to the attenuation of photosynthetically active radiation in some inland and coastal south-eastern Australian waters. Aust. J. Mar. Freshwater Res. 27: 61–71.

1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, New York, NY.

Kitchell, J. A., and J. F. Kitchell. 1980. Size-selective predation, light transmission and oxygen stratification: evidence from the recent sediments of manipulated lakes. Limnol. Oceanogr. 25: 389–402.

Kramer, J. R. 1978. Acid precipitation. *In* J. O. Nriagu [ed.] Sulfur in the environment: Part 1. The atmospheric cycle. John Wiley & Sons, New York, NY.

Kwiatkowski, R., and J. Roff. 1976. Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Can. J. Bot. 54: 2546–2561.

McAvoy, D. C., and C. T. Driscoll. 1989. Chemical response following base application to Little Simon Pond, New York State. J. Water Pollut. Control Fed. 61: 1552–1563.

Porcella, D. 1987. Overview of approach and liming of lakes for the Lake Acidification Mitigation Project (LAMP). Lake Reserv. Manage. 3: 401–403.

1989. Lake Acidification Mitigation Project (LAMP): an overview of an ecosystem perturbation experiment. Can. J. Fish. Sci. 46: 246–248.

Raddum, G., A. Hobaek, E. Lomsland, and T. Johnsen. 1980. Phytoplankton and zooplankton in acidified lakes in southern Norway, p. 332–333. *In* D. Drablos and A. Tollan [ed.] Ecological impact of acid precipitation. SNSF Project, Oslo, Norway.

Schaffner, W. R. 1989. Effects of neutralization and addition of brook trout (*Salvelinus fontinalis*) on the limnetic zooplankton communities of two acidic lakes. Can. J. Fish. Aquat. Sci. 46: 295–305.

Schafran, G. C., and C. T. Driscoll. 1987. Generation of acid neutralizing capacity in the hypolimnetic sediments of an acidic Adirondack lake. Envron. Sci. Technol. 221: 988–993.

Schindler, D. W., K. H. Mills, D. F. Malley, D. L. Findlay, J. A. Shearer, I. J. Davies, M. A. Turner, G. A. Linsey, and D. R. Cruikshank. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

Schofield, C. L. 1972. The ecological significance of air pollution induced changes in water quality and dilute lake districts in the northeast. Trans. N.E. Fish Wildl. Conf. p. 98–112.

Shearer, J. A., E. J. Fee, E. R. DeBruyn, and D. R. DeClercq. 1987. Phytoplankton primary production and light attenuation responses to the experimental acidification of a small Canadian Shield Lake. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 83–90.

Staubitz, W. W., and P. J. Zarriello. 1989. Hydrology of two headwater lakes in the Adirondack mountains of New York. Can. J. Fish. Aquat. Sci. 46: 268–276.

Wetzel, R. G. 1983. Limnology. Saunders College Publishing, Chicago, IL. p. 261.

Yan, N. D. 1983. Effects of changes in pH on transparency and thermal regimes of Lohi Lake, near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 40: 621–626.

Yan, N. D., and P. J. Dillon. 1982. Experimental neutralization of lakes near Sudbury, Ontario, chap. 6, p. 30–35. *In* Studies of lakes and watersheds near Sudbury, Ontario: Final limnological report. Ontario Ministry of the Environment, SES 009/82.

1984. Experimental neutralization of lakes near Sudbury, Ontario. *In* J. Nriagu [ed.] Environmental impacts of smelters. John Wiley and Sons, Toronto, Ont.

Effects of Elevated Winter Temperature on Seawater Adaptability, Sexual Rematuration, and Downstream Migratory Behaviour in Mature Male Atlantic Salmon Parr (*Salmo salar*)

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In experiments with two different salmon (*Salmo salar*) stocks, elevated winter temperatures (Celsius) 4–9° above the ambient increased the degree of testicular resorption in previously mature male Atlantic salmon parr. Two-summer-old mature male parr maintained at a temperature 9° over the ambient for 5 mo (January–May) showed an increase in hypoosmoregulatory ability when challenged in seawater at the time of smoltification whereas parr kept at elevated temperature for various 2-mo periods (January–February, February–March, or March–April) failed to show this improvement. Furthermore, previously mature males kept at an elevated winter water temperature for either 2 or 5 mo showed a lower incidence of sexual rematuration, and consequently a higher mean growth rate, after one summer in sea pens. A river release experiment showed that rearing previously mature males at 4–7° above the ambient water temperature from December to April increased the number of downstream migrating fish to a level similar to that of immature smolts. We suggest the use of a simple method for enhancing the potential yield of ranched and cultured salmon from early maturing males by removing them from the stock population and rearing them over winter in heated water.

Des expériences menées sur les saumons (*Salmo salar*) de deux stocks différents, ont permis de constater que l'élévation de la température (Celsius) hivernale de 4–9° au delà de la température ambiante détermine l'augmentation de la résorption testiculaire chez le tacon mâle du saumon de l'Atlantique ayant auparavant atteint la maturité. Des tacons mâles parvenus à maturité depuis deux étés ont été gardés pendant 5 mo (janvier–mai) à une température dépassant de 9° la température ambiante: on a constaté une augmentation de la capacité hypoosmorégulatrice lorsqu'on les placés en eau salée pour une provocation à l'époque de la smoltification; par contre, chez des tacons gardés à température élevée pendant 2 mo à diverses époques de l'année (janvier–fevrier, février–mars ou mars–avril), on n'a vu aucune amélioration comparable. En outre, chez des mâles matures gardés pendant 2 ou 5 mo à une température élevée par rapport à la température de l'eau en hiver, l'incidence de rematuration sexuelle était moindre, et par conséquent le taux de croissance moyen plus élevé, après un été en parc marin. Un lâcher expérimental en rivière a montré qu'en gardant des mâles matures à une température de 4–7° supérieure à la température de l'eau ambiante de décembre à avril, le nombre de poissons migrant vers la mer équivalait à peu près à celui des smolts immatures. Nous proposons une méthode simple pour améliorer le rendement potentiel de l'élevage extensif en mer et de l'élevage en parc : il s'agit de retirer les mâles en début de maturation de la population d'élevage et de leur faire passer l'hiver en eau réchauffée.

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As with many other salmonids, male Atlantic and Baltic salmon parr (*Salmo salar*) may become sexually mature in autumn (Mitans 1973; Thorpe and Morgan 1980; Saunders et al. 1982; Dalley et al. 1983; Lundqvist 1983). Parr that mature often show a poor hypoosmoregulatory capacity the following spring compared with nonmaturing fish (Lundqvist et al. 1989). They also show a higher preference for migrating upstream (Lundqvist and Eriksson 1985), and in wild stocks, few migrate seawards (Österdahl 1969). Previously mature male parr from hatchery stocks released into the river during the smolt migration are less likely to migrate to sea (Hansen et al. 1989), and they contribute significantly less to the sea catch of salmon than do previously immature fish (Eriksson et al. 1987; Lundqvist et al. 1988). Mature parr which were stripped of milt in autumn were 20% more likely to migrate the following spring than were those that had not been stripped (Hansen et al. 1989).

The physiological mechanism preventing the previously mature males from adopting a successful migratory strategy is not fully understoood. As Ikuta et al. (1985) had found with masu salmon (*Oncorhynchus masou*), so Lundqvist et al. (1989) showed with Atlantic salmon that nonresorbed gonads and/or relatively high gonadal steroid levels impaired seawater adaptability among young males. Previously mature males which migrated seaward had resorbed their gonads completely, and their gonadosomatic index (GSI) was usually <1%. The rate of gonadal resorption was temperature dependent and followed the seasonal temperature cycle. In the cold rivers of Scandinavia with winter temperatures often less than 2°C until April, the gonads were not completely resorbed until May, or even until June in northern rivers. Knowledge of the role of temperature in the control of smolting and gonadal resorption processes should help in understanding the regulatory mechanisms determining life history tactics.

The aim of the present study on previously mature male salmon parr was to test the effects of increased water temperature in winter and spring on gonadal resorption and on the subsequent development of hypoosmoregulatory capacity and readiness to migrate seawards. In addition the effects of thermal treatments on growth and rematuration rate during the first summer in a net pen in brackish water were studied.

Material and Methods

Fish and Holding Conditions

For each of the two salmon stocks, two-winter-old previously mature males and immatures of both sexes were sampled randomly from stock tanks where they had been kept at ambient water temperatures under normal production conditions. In the present paper, previously mature parr refer to male parr that matured sexually after their second summer and were classified as running ripe in the autumn. Parr from one Swedish stock Umeälven (Ume River) population were kept at the Norrfors Hatchery (63°50′N). Parr from the River Imsa stock were reared at the Research Station for Freshwater Fish at Ims (58°50′N), Norway. The normal smolting periods for the two stocks are the first week of June and the first two weeks of May, respectively.

Preliminary work at the Salmon Research Institute at Älvkarleby, Sweden, suggested that treatment of mature parr in elevated temperature for 3 wk had no effect on seawater adaptability (I. Berglund and H. Lundqvist, unpubl. data). We therefore used longer treatment periods in the present study.

Experimental Designs

Effect of exposure period on seawater adaptation and subsequent performance in net pens

The following null hypotheses were tested: 2- or 5-mo periods of increased temperature during winter have no effect on gonadal resorption, plasma steroid levels, or development of hypoosmoregulatory capacity in mature male salmon parr, nor on their subsequent growth and sexual maturation after transfer to net pens in brackish water.

At the Norrfors Hatchery, three groups of previously mature males ($n = 200$ per group) were reared at 9°C for 2-mo periods starting at 23 December, 23 January, and 23 February (1987–88), respectively, and then over 24 h gradually acclimated to ambient water temperature in which they were kept until late May. A fourth group was reared at a constant 9°C from 23 December to 23 May (5 mo), and a fifth untreated group kept at ambient temperature throughout the experiment served as a control. The water temperature in the ambient river water was <0.06°C from 23 December to 15 April when it slowly began to rise. It reached 6.6°C on 23 May when the fish in the 5-mo treatment were transferred to ambient temperature and 11°C on 1 June when the seawater challenge test was done (see below). Samples of 50 fish from each group were weighed and measured each month. The rate of gonadal resorption was estimated from GSI values of 10 fish that were killed at each sampling. On 1 June, 20 individuals selected at random from each group were challenged in 25 ppt seawater for 24 h to assess their seawater adaptation (Clarke and Blackburn 1977). It would have been preferrable to test the fish at several times during spring, but it was not possible to keep the required number of fish in our holding facilities. Therefore we did the single test close to the normal time for the smolt run in Umeälven. At the same time, 10 fish were sampled at random for subsequent analysis of plasma levels of testosterone (T), 11-ketotestosterone (OT), and 17α,20β-dihydroxyprogesterone (20P) at Bochum, BRD. It was not possible to detect plasma steroid levels below 0.3 ng/mL with the RIA technique used (Schulz 1985).

In addition, two control groups, immature and previously mature males, kept in ambient river temperature were followed through the smolting period to determine the seasonal pattern and normal level of seawater adaptation in the year of the experiment. A full report on this study is given in Lundqvist et al. (1990).

On 31 May the remaining fish (60–101 per group) were individually injected with passive integrated transponder (PIT) tags (Prentice et al. 1988) and transferred to a 100-m^3 net pen in the

Bay of Bothnia at Furugrund (64°55′N, 2–4 ppt brackish water). They were mixed with 500 untagged salmon smolts and fed a commercial dry food at 2–3% body weight per day. On 12 July, they were measured. At the end of the experiment on 29 September, they were weighed and measured, killed, and their degree of rematuration determined by examining the gonads.

The experiment was originally designed to investigate the effects of starting times of 2-mo thermal treatments. However, there were no significant differences between groups with different starting dates when final size and GSI after thermal treatment were taken into account, nor in growth or rematuration rate after transfer to net pens. All 2-mo groups performed poorly in the seawater challenge test with plasma sodium values at 202, 207, and 225 mM, respectively; the highest value was significantly different from the lowest, but no value was different from the ambient control and all values were close to that of the seasonal curve for mature males at that time. Because there appeared to be no consistent effects of the starting date of 2-mo treatments, the results from 2-mo treatments were pooled to test for different effects of 2- and 5-mo treatments. The Tukey HSD test was used to test for differences between the three resulting groups.

Effect on seaward migration

The following null hypotheses were tested: rearing temperature from November to April has no effect on gonadal resorption in mature male parr, nor on their probability of downstream migration at smolting time in May.

Sibling smolts of both previously mature males and immature fish of both sexes were reared at 7–12°C (4–7° above ambient water temperatures) for 4 mo from 17 December 1987 to 12 April 1988 at the research station at Ims. A control group was held at ambient temperatures throughout the same period. The ambient winter water temperature was around 2°C until late March when it started to rise and reached 10°C at release on 9 May. The fish in the heated group were transferred to ambient water temperature (6°C) 2 wk before release. In December, the mature males were easily classified by the presence of running milt.

The rate of migration of the different smolt groups was tested by releasing the Carlin-tagged smolts at the outlet of Lake Liavatn, 1000 m above the smolt trap in the River Imsa. The trap was monitored every 0.5 h the first night after release. Thereafter the trap was checked every second hour, and after 84 h twice a day. For each fish the time of capture was recorded, with their fork length (0.1 cm), weight (0.1 g), sex, and gonadal weight (0.1 g). Four days after release, the stocking site was electrofished and the nonmigratory fish were killed and examined.

Results

Seawater Adaptation and Performance in a Net Pen

Among the hatchery control fish at ambient temperature the immature males developed hypoosmoregulatory capacity in June and peaked around 9 June at 167 mM plasma sodium (Fig. 1). This year the plasma sodium curve of the previously mature males did not develop the typical minima they often do during spring (Lundqvist and Eriksson 1985; Lundqvist et al. 1989). The mean GSI of the previously mature males was 3.6% on 4 May and dropped to 0.8% on 22 June.

In the heated water experiment, fish kept at 9°C for 5 mo grew significantly faster than those from all other groups, reaching 55.7 g compared with 35.5 g for control fish (Table 1). Those kept at 9°C for 2 mo had a final mean weight of 38.5 g, significantly higher than the control fish ($p < 0.001$). At the end of the thermal treatment the GSIs were significantly lower after the longer treatment period (Table 2, $p < 0.001$).

In the seawater challenge test on 1 June, only those fish kept in heated water for 5 mo showed any increased hypoosmoregulatory capacity (Fig. 1). Their mean plasma sodium value close to 170 mM was significantly lower than for any other group ($p < 0.001$) and close to the seasonal curve of immature males at that time. Fish given thermal treatment for 2 mo did not differ in hypoosmoregulatory ability from the group kept at ambient temperature. Both groups performed poorly in seawater, with plasma sodium levels around that of the seasonal curve of mature males at that time. The difference between the 5-mo group and the other two groups was still significant after correction for weight differences among groups (ANCOVA, $p = 0.001$), but there was also a significant effect of weight ($p < 0.001$).

At the end of the experiment the plasma steroid content was unrelated to previous thermal treatment. On 1 June, all three steroids were below or close to the detection limit of the RIA technique used.

After one summer in net pens the 5-mo heated fish with the previously lowest GSI and best performance in seawater challenge tests were heavier than those of all other groups (Table 3, $p < 0.001$). However, ANOVA revealed no general effect of previous thermal treatment on the growth rate in the sea, but a significant difference between fish that matured sexually and those that did not ($p < 0.001$). There was, however, a tendency that rematuring males from the heated groups grew faster than those in the control group.

Sexual rematuration was high at 84.1% in the control, significantly higher (multiple comparison test for proportions (Zar 1987), $p < 0.001$) than in all other groups (28.9 and 38.7%) which did not differ statistically from one another (Fig. 2). At the time of sea transfer the individuals that stayed immature within a treatment group were slightly longer (mean difference = 3.8 mm) than those that matured again (ANOVA, $p = 0.034$), but they did not differ in weight.

The combination of a low number of rematuring fish and a higher final weight for both life history types resulted in a mean weight of 196 g for fish kept at 9°C for 5 mo compared with 94.8 g for the control, i.e. 107% higher production of biomass in the 5-mo group and a considerably higher proportion of more valuable nonrematuring fish (Fig. 2).

During the time in the net pen, 14 fish were found dead, and unfortunately a considerable number of fish were missing. We assume that these fish escaped during an accident with the floating device of the net pen.The proportion of missing fish differed among groups (31.0, 40.0, and 54.4%), with the lowest percentage in the control and the highest among fish heated for 5 mo, which had the largest and most seawater-adapted fish at sea transfer. There were no significant differences in length or weight at the start of the experiment between fish that were missing or present at the end of the experiment (ANOVA, $p > 0.05$).

Seaward Migration

All categories of smolts kept in heated water descended to the trap more quickly than corresponding unheated groups (Fig. 3, Kolmogorov–Smirnov test, $p < 0.001$). Among previously

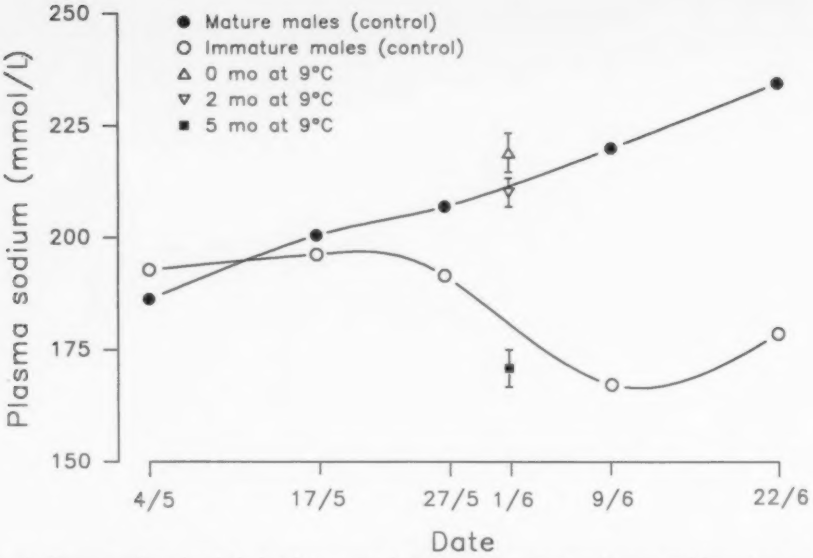

FIG. 1. Plasma sodium levels in hatchery controls (previously mature male parr and immature male parr) kept at ambient temperature and previously mature males given thermal treatments at 9°C for 0, 2, or 5 mo. The curves showing the seasonal changes in hatchery controls are revised (only mean values for males shown) from Lundqvist et al. (1990). Means and standard errors are shown for groups given thermal treatment ($n = 15$–20 in treatment groups).

TABLE 1. Initial (23 December) and final (31 May) mean size (SD in parentheses) of previously mature male parr given thermal treatment in the experiment on seawater adaptability and performance in a net pen.

Group[a]	Initial mean size[b]		Final mean size[c]	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
0 mo	14.8 (1.1)	38.5 (8.6)	14.7 (0.8)	35.5 (5.8)
2 mo	14.9 (1.0)	38.6 (8.1)	15.5 (1.0)	38.5 (7.3)
5 mo	14.9 (1.1)	38.4 (8.1)	17.1 (1.1)	55.7 (8.8)

[a] Months at elevated (9°C) water temperature.
[b] Based on samples of 50 fish.
[c] Based on 60–256 fish, transferred to a net pen.

TABLE 2. Mean size and GSI (SD in parentheses, n (fish surviving in test) = 15–20) of previously mature male parr subjected to seawater challenge tests after thermal treatments at 9°C for 0, 2, or 5 mo.

Group	Length (cm)	Weight (g)	GSI (%)
0 mo	14.0 (1.0)	27.6 (5.7)	1.9 (0.6)
2 mo	15.2 (1.0)	33.8 (7.1)	0.7 (0.1)
5 mo	17.2 (1.4)	51.5 (11.5)	0.2 (0.1)

TABLE 3. Mean initial and final weight (SD in parentheses) and growth rate of immature and maturing salmon males kept one summer in a net pen in brackish water. The fish transferred to the sea were previously mature male parr that had been given thermal treatment at 9°C for 0, 2, or 5 mo.

Group	Category	n	Initial weight (g)	Final weight (g)	Growth rate (%/d)
0 mo	Immature	11	37.4 (6.38)	143.3 (45.0)	1.08 (0.22)
	Mature	58	34.7 (6.03)	85.7 (17.8)	0.74 (0.12)
2 mo	Immature	110	38.1 (6.16)	150.5 (50.8)	1.10 (0.22)
	Mature	42	37.9 (9.05)	107.0 (32.3)	0.84 (0.14)
5 mo	Immature	19	56.4 (9.14)	213.5 (69.5)	1.08 (0.19)
	Mature	12	54.8 (10.7)	168.3 (59.6)	0.89 (0.22)

mature males the proportion which migrated was higher for the heated fish (Table 4, G-test, $p < 0.05$). At release, immatures were significantly larger than mature males kept at the same temperature, (Table 5, t-tests, $p < 0.01$). Immatures reared in heated water were larger than those in ambient temperature ($p < 0.01$), but not so for the mature males ($p > 0.05$). GSIs were smaller for heated than control fish (t-test on arcsin-transformed data, $p < 0.001$). The testes of 35% of the heated previously mature males were heavily pigmented with melanin, but none of the controls were.

Of the 24 fish sampled at the site of release 4 d after, 22 were previously mature males, 9 of which had been reared in heated water and 13 of which were controls reared at ambient temperature. The mean GSI of heated residents was significantly higher than that of the migrants (Table 6, Mann–Whitney U-test, $p < 0.001$), but not so among the controls ($p > 0.05$). Furthermore, the mean GSI of the migratory males kept at ambient temperature did not differ from that of the resident males in the heated group.

Discussion

Summary of Effects of Thermal Treatment

Temperatures higher than 4° over ambient winter temperature increased the degree of testicular resorption in previously mature male parr in experiments at all sites. Thermal treatment at 9° over ambient temperature during the entire period of December–May increased the hypoosmoregulatory ability in mature parr whereas heating during various 2-mo periods did not have this effect. Furthermore, previously mature males heated 2 or 5 mo showed a lower incidence of sexual rematuration after one summer in sea pens. Apart from the

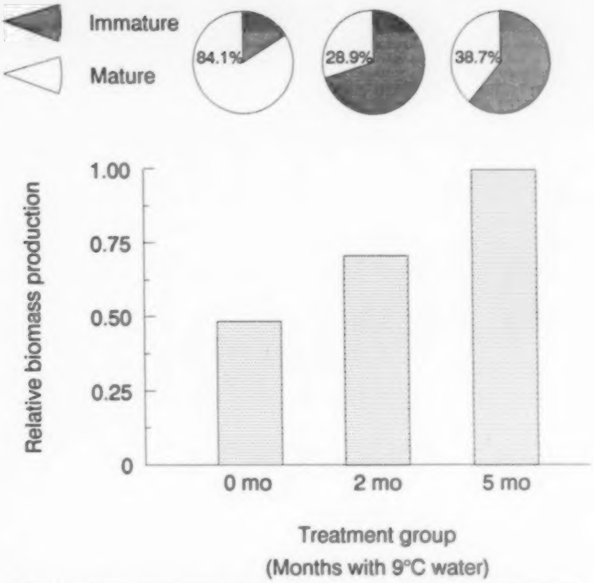

FIG. 2. Sexual rematuration rate (pies) and relative biomass production (relative mean weight at the end of the experiment) of previously mature male parr kept in net pens in brackish water from 1 June to 29 September. Prior to sea transfer the fish had been given thermal treatment at 9°C for 0, 2, or 5 mo.

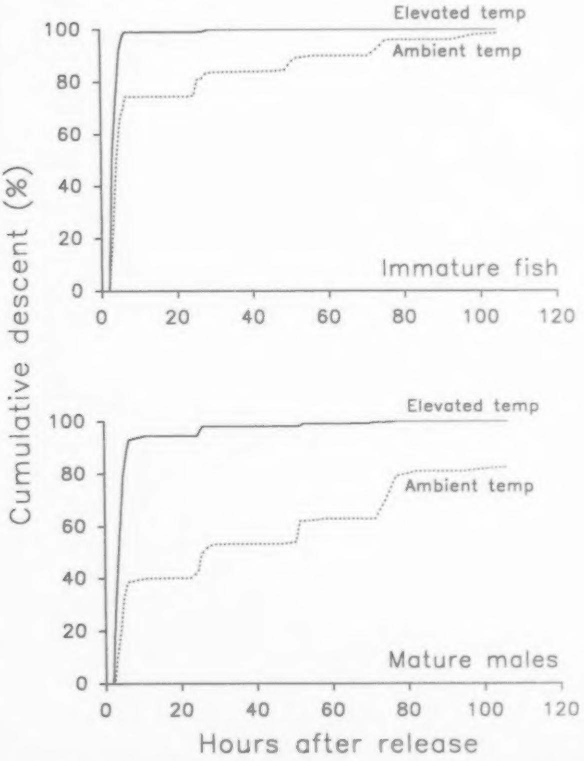

FIG. 3. Cumulative percent of descending smolts from immature parr (upper) and previously mature male parr (lower) reared at ambient or elevated winter water temperature.

TABLE 4. Number of released and migrant smolts from immature parr (I) and mature male parr (M) in the experiment on seaward migration. Prior to release on 9 May the fish had been kept in ambient temperature or heated water from 17 December to 12 April. The fish were released 1000 m above the smolt trap in the River Imsa.

Group	No. released	No. migrants	Migrants(%)
Heated M	210	171	81.4
Ambient M	210	122	58.1
Heated I	239	206	86.2
Ambient I	235	207	88.1

TABLE 5. Mean length of immature parr and sexually mature male parr at release in the River Imsa on 9 May. The fish had been kept in ambient temperature or heated water from 17 December to 12 April.

Temperature	Maturity stage	*n*	Mean length (cm)	SD
Ambient	Immature	27	21.1	5.6
	Mature males	23	17.1	3.4
Heated	Immature	25	26.2	6.7
	Mature males	25	18.5	2.4

TABLE 6. Mean GSI of resident and migratory previously mature parr released in the River Imsa on 9 May. Prior to release the fish had been kept in ambient temperature or heated water from 17 December to 12 April.

Temperature	Group	*n*	Mean GSI(%)	SD
Ambient	Resident	13	1.447	0.944
	Migratory	23	1.108	0.569
Heated	Resident	8	0.957	0.494
	Migratory	37	0.326	0.350

physiological aspect of smolting, the release experiment showed that rearing previously mature males in heated water during winter also affected behavioural aspects of smolting. Maintaining mature males in heated water increased the number of downstream-migrating fish to a level similar to that of immature fish.

Biological Significance

Saunders et al. (1982) kept mature male parr elevated (10°C) and ambient temperature (minimum 3.4°C in March) from December to April. They found a faster decline of the GSI at the higher temperature. However, in contrast with Langdon and Thorpe (1985), Birt and Green (1986), and Lundqvist et al. (1989), Saunders et al. (1982) did not find any consistent difference in seawater tolerance between previously mature and immature parr. Furthermore, they found no effect of temperature treatment on salinity tolerance in mature parr, except that the salinity tolerance developed earlier at higher temperature.

Nagahama et al. (1982) found low levels of plasma T in immature amago salmon (*Oncorhynchus rhodurus*), and Patino and Schreck (1986) found low levels of OT and 20 P in juvenile coho salmon (*O. kisutch*) during their smolting periods. Furthermore, Mayer et al. (1990) found no consistent differences in androgen levels in mature and immature fish at the time of smoltification. Castration of previously mature Baltic salmon parr in the spring 2.5 mo before smolting had no effect on seawater adaptation (SWA) (Lundqvist et al. 1990) whereas castration in autumn had a positive effect on SWA in masu salmon (Aida et al. 1984). However, it has been shown that the administration of androgens impairs SWA in a variety of salmonids (Miwa and Inui 1986; Ikuta et al. 1985; Lundqvist et al. 1989). Accordingly, Lundqvist et al. (1990) concluded that the difference in SWA between previously mature males and immature fish in Baltic salmon was more likely a persisting effect from the high androgen levels in the previous breeding season than due to the presence of gonads and plasma androgens at the time of smolting in spring. Therefore we conclude that the positive effect of long-term temperature treatment on osmoregulation does not result from increased rate of gonadal resorption per se. Rather, the effect appears to be due to an increased metabolic rate at higher temperatures, which reverses the negative effects of the previous maturation on SWA.

This view is also supported by the results from the river release experiment at Ims where the mean GSI of the migratory males in the group at ambient temperature did not differ from that of the resident males in the heated group. Accordingly, it seems unlikely that the increased number of migratory fish was dependent on the size of the gonads at that time.

Hypoosmoregulatory ability, as determined in seawater challenge tests, is also likely to be influenced by fish size (Lundqvist et al. 1986). Since the thermal treatment in the present experiment caused differences in fish size, it is difficult to distinguish between the effect of size and other physiological effects caused by the treatment. However, separation of size from other effects was attempted by including weight as a covariate in the analysis. The result of the ANCOVA showed that although the effect of fish weight on osmoregulation was significant, there were still significant differences among groups after correction for differences in weight.

Even though previously mature male parr show poorer hypoosmoregulatory ability compared with immature fish when challenged with seawater, both groups usually show optimal SWA at the same time (Clarke et al. 1985; Lundqvist et al. 1989). This temporal peak in hypoosmoregulatory ability may, however, be absent among the mature males (Lundqvist et al. 1990; see also Fig. 1). It has also been shown that elevated temperatures can accelerate the smolting process in salmonids (Zaugg and Mclain 1976; Saunders et al. 1982; Clarke et al. 1985). The seawater challenge test in the present study was performed only once, at a date (1 June) closely corresponding to the time for optimal SWA during smoltification for the stock used. From that single data point, it is difficult to judge if the time of optimal SWA was advanced compared with the control curve. However, the mean plasma sodium level of the fish kept at an elevated temperature for 5 mo was similar to that of immature control fish at that time, while those kept for 2 mo had sodium values not different from the group kept at ambient temperature. The fact that all heated groups were returned to ambient temperature before the fish were tested in seawater, together with the influence of the more powerful *zeitgeber*, the natural photoperiod, seems to have brought the smolting process of the heat-treated fish approximately into phase with the control fish.

The slower downstream movement of the groups kept at ambient temperature in the migration experiment may partly be due to their smaller body size, in agreement with previous results from the River Imsa. Both large wild (Jonsson et al. 1990) and hatchery-reared smolts (Hansen and Jonsson 1985; Hansen et al. 1989) migrate earlier in the spring and more quickly than smaller smolts. However, this may only be a part of the explanation, as immatures kept at ambient temperature descended more slowly than the smaller heated males. When salmon parr are exposed to warmer water during winter, they smolt earlier in spring, as stated above (Jonsson and Ruud-Hansen 1985). This implies that smolting is a gradual process and that more advanced smolts descend sooner.

The finding that individuals which remained immature after transfer to net pens were slightly larger at transfer compared with rematuring fish is consistent with the results presented by Eriksson et al. (1987) who found a higher tendency for rematuration among smaller fish after their first summer in net pens. The length difference between the two categories of fish in the present study was small and may have been due to faster growth in length, during smolting, among fish that later remained immature in the net pen.

The present results demonstrate that long-term thermal treatment of previously mature male salmon parr during winter–spring increases SWA and migratory capacity and decreases their propensity for sexual rematuration after transfer to net pens. We therefore suggest a simple method for enhancing the potential yield of ranched and cultured salmon smolts from early maturing males by removing them from the stock population and rearing them over winter in heated water. The precise period and amount of heating for most effective results have yet to be determined, but apparently the period must exceed 2 mo.

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References

Aida, K., T. Kato, and M. Awaji. 1984. Effects of castration on the smoltification of precocious male masu salmon (*Oncorhynchus masou*). Bull. Jpn. Soc. Sci. Fish. 50: 565–571.

Birt, T. P., and J. M. Green. 1986. Parr–smolt transformation in sexually mature male anadromous and nonanadromous Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 43: 680–686.

Clarke, W. C., and J. Blackburn. 1977. A seawater challenge test to measure smolting in juvenile salmon. Fish. Mar. Serv. Tech. Rep. No. 705.

Clarke, W. C., H. Lundqvist, and L.-O. Eriksson. 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol. 26: 29–35.

Dalley, E. L., C. W. Andrews, and L. M. Green. 1983. Precocious male Atlantic salmon parr (*Salmo salar*) in insular Newfoundland. Can. J. Fish. Aquat. Sci. 40: 647–652.

Eriksson, T., L.-O. Eriksson, and H. Lundqvist. 1987. Adaptive flexibility in life history tactics of mature male Baltic salmon (*Salmo salar*) parr in relation to body size and environment, p. 236–245. *In* M. J. Dadswell, R. J. Klauda, C. M. Moffitt, R. L. Saunders, R. A. Rulifson, and J. E. Cooper [ed.] Common strategies of anadromous and catadromous fishes: Proceedings of American Fisheries Society Symposium No. 1. American Fisheries Society, Bethesda, MD.

Hansen, L. P., and B. Jonsson. 1985. Downstream migration of hatchery reared smolts of Atlantic salmon (*Salmo salar* L.) in the River Imsa, Norway. Aquaculture 45: 237–248.

Hansen, L.-P., B. Jonsson, R. I. G. Morgan, and J. E. Thorpe. 1989. Influence of parr maturity on emigration of smolting Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 46: 410–415.

Ikuta, K., K. Aida, N. Okumoto, and L. Hanyu. 1985. Effects of sex steroids on the smoltification of masu salmon (*Oncorhynchus masou*). Gen. Comp. Endocrinol. 65: 99–110.

Jonsson, B., and J. Ruud-Hansen. 1985. Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 42: 593–595.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1990. Partial segregation in timing of migration of Atlantic salmon of different age. Anim. Behav. 40: 313–321.

Langdon, J. S., and J. E. Thorpe. 1985. The ontogeny of smoltification: developmental patterns of gill Na^+/K^+-ATPase, SDH, and chloride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture 45: 83–95.

Lundqvist, H. 1983. Precocious sexual maturation and smolting in Baltic salmon (*Salmo salar*); photoperiodic synchronisation and adaptive significance of annual biological cycles. Ph.D. thesis, University of Umeå, Umeå, Sweden. 75 p.

Lundqvist, H., I. Berglund, I. Mayer, and B. Borg. 1990. Seawater adaptability in Baltic salmon, *Salmo salar*, immature smolt and mature male parr: lack of effect of springtime castration. Can. J. Zool. 68: 2181–2184.

Lundqvist, H., B. Borg, and I. Berglund. 1989. Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). Can. J. Zool. 67: 1733–1736.

Lundqvist, H., W. C. Clarke, L.-O. Eriksson, P. Funegård, and B. Engström. 1986. Seawater adaptability in three different river stocks of Baltic salmon (*Salmo salar* L.) during smolting. Aquaculture 52: 219–229.

Lundqvist, H., W. C. Clarke, and H. Johansson. 1988. The influence of precocious sexual maturation on survival to adulthood of river stocked Baltic salmon, *Salmo salar*, smolts. Holarct. Ecol. 11: 60–69.

Lundqvist, H., and L.-O. Eriksson. 1985. Annual rhythms of swimming behaviour and seawater adaptation in young Baltic salmon, *Salmo salar*, associated with smolting. Environ. Biol. Fishes 14: 259–267.

Mayer, I., H. Lundqvist, I. Berglund, M. Schmitz, R. Schulz, and B. Borg. 1990. Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17β-estradiol. Can. J. Zool. 68: 1360–1365.

Mitans, A. R. 1973. Dwarf males and the sex structure of a Baltic salmon (*Salmo salar*) population. J. Ichtyol. 13: 192–197.

Miwa, S., and Y. Inui. 1986. Inhibitory effects of 17β-methyltestosterone and estradiol-17β on smoltification of sterilized amago salmon *Oncorhynchus rhodurus*. Aquaculture 53: 21–39.

Nagahama, Y., S. Adachi, F. Tahshiro, and E. G. Grau. 1982. Some endocrine factors affecting the development of seawater tolerance during the parr–smolt transformation of the amago salmon, *Oncorhynchus rhodurus*. Aquaculture 28: 81–90.

Österdahl, L. 1969. The smolt run of a small Swedish river, p. 205–215. *In* T. G. Northcote [ed.] Salmon and trout in streams. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C.

Patino, R., and C. B. Schreck. 1986. Sexual dimorphism of plasma sex steroid levels in juvenile coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol. 61: 127–133.

Prentice, E. F., T. A. Flagg, and C. A. McCutcheon. 1988. Passive integrated transponder tag for fish. Proceedings for the American Fisheries Society, International Symposium and Educational Workshop on Fish-marking Techniques. American Fisheries Society, Bethesda, MD.

Saunders, R. L., E. B. Henderson, and B. D. Glebe. 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture 28: 211–229.

Schulz, R. 1985. Measurement of five androgens in the blood of immature and mature male rainbow trout, *Salmo gairdneri* (Richardson). Steroids 46: 717–726.

Thorpe, J. E., and R. I. G. Morgan. 1980. Growth-rate and smolting-rate of progeny of male Atlantic salmon parr, *Salmo salar*, L. J. Fish Biol. 17: 451–460.

Zar, J. A. 1987. Biostatistical analysis. Prentice-Hall, Engelwood Cliffs, NJ. 718 p.

Zaugg, W. S., and L. R. McLain. 1976. Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 35: 587–596.

Hypolimnetic O_2 Consumption, Denitrification, and Methanogenesis in a Thermally Stratified Lake

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Bédard, C., and R. Knowles. 1991. Hypolimnetic O_2 consumption, denitrification, and methanogenesis in a thermally stratified lake. Can. J. Fish. Aquat. Sci. 48: 1048–1054.

Hypolimnetic O_2 consumption, methanogenesis, and denitrification were estimated from concentration changes in O_2, CH_4, and nitrogen oxides (NO_3^-, NO_2^-, N_2O), respectively, in the eastern basin of Lake St. George, Ontario, during thermal stratification from 1980 to 1984. NH_4^+ accumulated in the hypolimnion throughout the period of stratification and provided an overall measure of organic matter mineralization. Anaerobic metabolism, particularly methanogenesis, predominated because anoxia was total within less than 2 mo of spring water column turnover and nitrogen oxides were always completely depleted before fall turnover. The time of onset and rate of NO_3^- depletion varied considerably between years and were not always directly correlated with O_2 concentration. It was estimated that, on average, O_2 consumption, denitrification, and methanogenesis accounted for approximately 80% of hypolimnetic organic C mineralization in the periods surveyed.

Au cours de la stratification thermique du bassin oriental du lac St. George (Ontario) pendant la période 1980–1984, on a déterminé le taux de consommation de O_2, la méthanogénèse et la dénitrification dans l'hypolimnion à partir des variations des teneurs en O_2, CH_4 et en oxides d'azote (NO_3^-, NO_2^- et N_2O), respectivement. Le NH_4^+ accumulé dans l'hypolimnion pendant toute la période de stratification a servi de mesure globale de la minéralisation de la matière organique. Le métabolisme anaérobie, en particulier la méthanogénèse, prédominait étant donné que l'anoxie était complète en-déçà de 2 mo du brassage convectif de la colonne d'eau au printemps et que les oxides d'azote étaient complètement épuisés avant le brassage convectif d'automne. Le début de l'appauvrissement en NO_3^-, et le taux d'appauvrissement, variaient fortement d'une année à l'autre et n'étaient pas toujours directement en corrélation avec la teneur en O_2. En moyenne, la consommation de O_2, la dénitrification et la méthanogénèse expliquent environ 80 % de la minéralisation du C organique dans l'hypolimnion pendant la période expérimentale.

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The hypolimnion of thermally stratified lakes is an active site for organic matter mineralization as evidenced by the decrease in concentration of various substrates for microbial respiration, including O_2, NO_3^-, and SO_4^{2-}, as well as the accumulation of metabolic end-products such as NH_4^+, CH_4, and H_2S (Kelly et al. 1982; Wetzel 1983). Hypolimnetic O_2 utilization has been intensively investigated (Hutchinson 1957; Cornett and Rigler 1980). In addition, attention has been directed to some anoxic mineralization processes, e.g. methanogenesis (Strayer and Tiedje 1978; Rudd and Hamilton 1978; Kelly and Chynoweth 1980; Fallon et al. 1980; Kelly et al. 1982). However, there appear to have been fewer attempts to measure several processes simultaneously and to determine their relative contribution to total hypolimnetic metabolism (Kelly et al. 1982; Brock 1985).

An earlier technical report (Knowles et al. 1990) noted marked changes in hypolimnetic concentrations of O_2, various oxides of nitrogen, CH_4, and NH_4^+ in the two basins of mesotrophic Lake St. George, Ontario, during the period of thermal stratification in several years. In most years, hypolimnetic O_2 is rapidly and completely depleted. Anaerobic processes predominate during the remainder of the period of stratification. One objective of the present work was to describe the temporal pattern of change of the various chemical species mentioned above in the larger and deeper of the two basins for which a more complete data set was available. Using these data, we have estimated areal hypolimnetic rates of O_2 respiration, denitrification, methanogenesis, and total organic C mineralization. For each process, the relative contributions of changes measured within the hypolimnion and vertical mass transfer across the upper boundary of the hypolimnion were examined. We speculated that vertical transport of oxidant across this boundary might contribute significantly to estimates of total hypolimnetic O_2 respiration and denitrification because O_2 and NO_3^- are completely depleted from the hypolimnion long before the end of thermal stratification. The relative importance of the various processes of organic C mineralization taken alone or together to total organic C mineralization was considered. We expected that because O_2 is absent from the hypolimnion for more than two thirds of the period of stratification, a major proportion of the organic C mineralization occurring during this period should proceed via anaerobic pathways. Finally we note the existence of sometimes considerable interannual variation in some of the mineralization processes measured; this variation cannot be readily explained by available data. This investigation is complementary to earlier studies on Lake St. George sediment and water samples in which denitrification (Chan and Knowles 1979; Knowles 1979), nitrification (Knowles and Lean 1987), and CH_4 production (Knowles 1979) were investigated.

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Methods

Lake St. George (43°57'30"N, 79°25'30"W) is 2 km east of Oak Ridges and about 32 km north of Toronto, Ontario. Its location on a height of land, with no major inflow, minimizes pollution from upstream sources. It is a mesotrophic double-basin lake (10.3 ha) described by Knowles and Lean (1987). Knowles et al. (1990) presented a compilation of raw data for the lake between 1976 and 1984 which forms the basis of the present report. This study was carried out in the 5.88-ha eastern basin of the lake which has a maximum depth of 17 m and which undergoes pronounced seasonal changes in forms of N (Knowles et al. 1981). NO_3^- concentrations range from near zero to 114 μM, and CH_4 accumulations can reach 500 μM in the hypolimnion during summer stratifications.

Samples were taken and processed as described in Knowles and Lean (1987). The methods for the measurement of temperature, O_2, and N fractions were described by Knowles et al. (1981). Dissolved CH_4 was determined as for N_2O except that the gas chromatographic system (Perkin Elmer 3920) consisted of a 200 cm × 3.3 mm column of Porapak N at a temperature of 65°C with He carrier gas and a hydrogen flame ionization detector.

Total hypolimnetic mass of O_2, NH_4^+, NO_2^-, NO_3^-, N_2O, and CH_4 (8–15 m) were calculated by trapezoidal integration for the whole lake basin based on data for concentration and volume per layer (see Knowles and Lean 1987 for an example). The rate of change in each chemical species was determined by linear regression of hypolimnetic mass against time.

Material flux across the upper boundary of the hypolimnion was estimated using Fick's Law:

$$F = -D(dc/dx).$$

Concentration gradients (dc/dx) in the 5- to 8-m depth range on each sampling date were calculated. An average dc/dx value for the period between two consecutive dates was derived and used to obtain an average daily flux for that period of time. Total fluxes were computed for each period and summed to yield an overall value for the period of thermal stratification. Lower and upper flux estimates were calculated using diffusion coefficients (D) (Quay et al. 1980) of 5×10^{-5} (Lake 227) and 8×10^{-4} (Lake 224) $cm^2 \cdot s^{-1}$, respectively.

Results

Following ice break-up, water column turnover saturated the hypolimnion of Lake St. George with O_2 during mid-April in all years surveyed, except 1984 (Fig. 1). O_2 depletion began immediately after spring turnover and was complete by late May or early June. Hypolimnetic O_2 levels in the spring of 1984 remained close to their low winter values (Knowles et al. 1990), providing evidence for the near or total absence of water column overturn. As will be seen below, further evidence for the lack of spring mixing in 1984 comes from hypolimnetic levels of other chemical species studied here. Hypolimnetic O_2 depletion in 1984 was not complete until July. Reasons for the persistence of measurable levels of O_2 in this year are not readily apparent.

Temporal patterns of fluctuation in hypolimnetic NO_3^-, NO_2^-, and N_2O suggest denitrification (Fig. 1). In 1982, 1983, and 1984, the years for which nitrogen oxide data sets are most complete, a clear temporal succession of NO_3^-, NO_2^-, and N_2O peaks occurred. Mean peak values of NO_2^- and N_2O for the three years were 67 ± 11% and 10 ± 5% of the NO_3^- peak value, respectively. The apparent lack of any increase in the rate of NH_4^+ accumulation during the period of NO_3^- and NO_2^- disappearance suggests that dissimilatory reduction of NO_3^- to NH_4^+ was not a major pathway of NO_3^- metabolism in the hypolimnion.

Decreases in hypolimnetic NO_3^- in the years surveyed began immediately following or as late as 2.5 mo after spring turnover (Fig. 1). Slight initial increases in NO_3^- occurred following turnover in 1980, 1982, and 1983. Nitrification may have been responsible for the increases, since O_2 was generally present at these times. However, during periods of NO_3^- increase, NH_4^+ also tended to accumulate. O_2 disappeared from the hypolimnion before NO_3^- in all years except 1984. Complete depletion of NO_3^- occurred as early as the beginning of June (1984) and as late as early September (1983) (Fig. 1). Although NO_3^- depletion generally began during the period when O_2 was being consumed, in 1980, the onset of NO_3^- disappearance occurred 1 mo after complete O_2 depletion. Subsequently, NO_3^- disappeared at the highest rate of any of the years surveyed (see Table 1). A puzzling feature of the 1980 data is the increase in both total hypolimnetic NO_3^- and NO_2^- observed between May 27 and June 24. The increase amounted to 41% (1.8 kmol) of the total N present as $NO_3^- + NO_2^-$ on May 27. The lack of O_2 in the hypolimnion over this period would appear to preclude nitrification as a source of nitrogen oxides.

In most years, production and consumption of hypolimnetic NO_2^- appeared to proceed simultaneously. However, in the period May 18 to June 29 in 1983, total hypolimnetic NO_3^- decreased by 1.6 kmol, while NO_2^- increased by an essentially equal amount (1.7 kmol), indicating stoichiometric conversion of NO_3^- to NO_2^-.

The peak accumulation of N_2O in 1984 was the earliest and highest observed in any year for which data are sufficiently complete. In other such years, N_2O accumulation generally appears to have occurred later in the summer in the complete absence of O_2. Interestingly, 1984 was the only year in which significant amounts of O_2 were present at the time of N_2O accumulation.

In most years, CH_4 began to increase during the period of NO_3^- depletion and after O_2 depletion (Fig. 1). In mid-April of 1984, because of the lack of turnover, most of the CH_4 produced during winter was still present in the hypolimnion (Knowles et al. 1990). In other years, CH_4 did not begin to increase significantly until the month of June.

Hypolimnetic NH_4^+ accumulation was generally continuous over the period mid-April to early fall (Fig. 1). Large-scale variations in the time courses are probably related to analytical problems. NH_4^+ levels in mid-April of 1984 were high relative to other years due, once again, to winter accumulation and the absence of spring turnover in this year.

The time courses of the total amounts of the various chemical species measured in the hypolimnion were approximately linear over at least part of the period examined (Fig. 1). It was therefore possible to estimate rates of change by linear regression (Sokal and Rohlf 1981). There are indications of a degree of nonlinearity in some of the depletion and accumulation curves (Fig. 1). For example, slowed rates of O_2 and NO_3^- con-

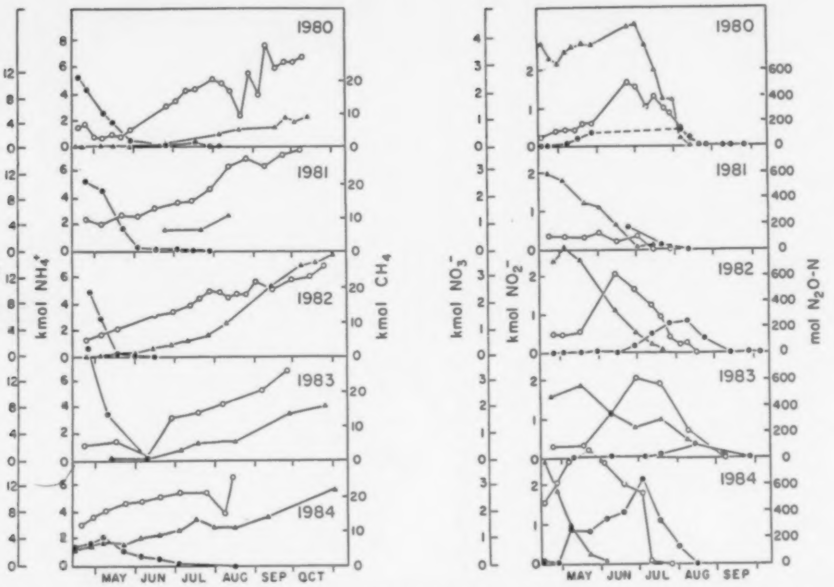

FIG. 1. Total hypolimnetic (8–15 m) contents of certain molecular species in Lake St. George in the years 1980–84. Left: O_2 (●), CH_4 (▲), and ammonia (○). Right: NO_3^- (▲), NO_2^- (○), and N_2O (●).

sumption near the end of the depletion period are hinted at and may indicate limitation due to low substrate concentration. However, the generally high (>0.90) and significant r^2 values obtained in cases where sufficient data points were available (Table 1) appear to justify the use of simple linear regression, at least for the purposes of the present report. Excluding 1984, rates of O_2 consumption did not vary by a factor of more than 2 between years whereas rates of NO_3^- and NO_2^- decrease varied by as much as five- and fourfold, respectively (Table 1). Interannual variation in rates of CH_4 accumulation was never greater than threefold. Excluding 1984, NH_4^+ accumulation rates varied by less than twofold. On average, NO_3^- disappeared approximately twice as fast as NO_2^-, and NO_2^- itself was consumed at 3 times the rate of N_2O (Table 1). These differences in disappearance rates could explain the succession of NO_2^- and N_2O peaks observed in several years (Fig. 1).

With the possible exception of O_2 in 1984, fluxes of chemical species into and out of the hypolimnion over the period of thermal stratification were approximately 1–3 orders of magnitude lower than changes in the amount of these species within the hypolimnion itself (Table 2). Therefore, even in the case of O_2 and NO_3^- which were generally quickly depleted from the hypolimnion, mass transfer across the thermocline did not contribute significantly to the mineralization processes measured here.

The amount of organic C mineralized in the hypolimnion by O_2 consumption, denitrification, and methanogenesis can be estimated using conversion factors in the literature (Table 3). The estimates of Table 3 suggest a number of trends. Methanogenesis predominated over the two other processes of mineralization. In the four years for which complete data are available, methanogenesis was responsible, on average, for approximately 70% of the organic C mineralized by the three processes combined (Table 3). On average, O_2 consumption accounted for nearly twice the mineralization due to denitrification (Table 3). In all years in which complete turnover of the water column occurred during spring (i.e. all years surveyed except 1984), O_2 consumption predominated over denitrification as a mineralization pathway (Table 3).

Two processes could account for NH_4^+ accumulation in the hypolimnion during thermal stratification: NO_3^- dissimilation to NH_4^+ and organic matter mineralization. However, as discussed above, the former process does not appear to be quantitatively significant. NH_4^+ accumulation may, therefore, be essentially a measure of total organic matter mineralization. This measure can be expressed in terms of organic C using an appropriate C:N ratio (Table 3).

Over the five years examined, mineralization due to combined O_2 respiration, denitrification, and methanogenesis did not strictly correlate with total mineralization values derived from NH_4^+ accumulation (Table 3). If, however, the average values of both mineralization estimates for the five years are considered (Table 3), it appears that O_2 respiration, denitrification, and methanogenesis may account for most of the total hypolimnetic organic matter mineralization. It should be emphasized that a number of assumptions underlie the estimates of Table 3. Most conversion factors used to express a mineralization process in terms of C were obtained from the literature. C:N ratios were derived from particulate organic C (POC) and total particulate N (TPN) data which were not complete for all years included in the present study (Knowles et al. 1990; see also Table 3). It is further assumed that mineralization leads to the regeneration of C and N in a ratio similar to that derived from particulate matter analyses. The intersection of CH_4 and NH_4^+ accumulation curves in 1982 (Fig. 1) indicates that, at some times, organic N mineralization may lag behind organic C mineralization. The total mineralization value in 1984 may represent an underestimate. The value was derived from the NH_4^+ accumulation rate estimate for that year (Table 1) because, unlike previous years, NH_4^+ concentration data at the end of the period of stratification were not available. The validity of the rate estimate is questionable due to the low r^2 and

TABLE 1. Average rates (mmol·m^{-2}·d^{-1}) of decrease of O_2, NO_3^-, NO_2^-, and N_2O and inc
the years 1980–84.

	Mean hypolimnetic temperature (°C)	O_2	NO_3^-	NO_2
1980				
Rate[a]	5.06 ± 0.03	22.5 ± 1.4	10.18 ± 0.81	2.24 ±
r^{2b}		0.99**	0.97**	0.89*
Period[c]	Apr. 23 – Oct. 9	Apr. 17 – May 27 (May 29)	July 11 – Aug. 12 (Aug. 10)	July 1 – A (Aug.
1981				
Rate	6.23 ± 0.09	24.3 ± 3.4	3.59 ± 0.24	0.67 ±
r^2		0.96**	0.98**	0.54 n
Period	Apr. 22 – Oct. 5	Apr. 22 – June 1 (June 4)	Apr. 22 – July 2 (July 7)	June 1 – J (July
1982				
Rate	4.97 ± 0.02	38.5 ± 5.54	4.94 ± 0.37	3.13 ±
r^2		0.98 n.s.	0.97**	0.97*
Period	Apr. 26 – Nov. 1	Apr. 26 – May 17 (May 18)	May 3 – July 26 (July 19)	June 14 – (Aug.
1983				
Rate	6.16 ± 0.06	32.10 ± 40.94	1.79 ± 0.33	2.82 ±
r^2		0.91 n.s.	0.88*	0.94*
Period	Apr. 25 – Oct. 18	Apr. 25 – June 9 (June 7)	May 18 – Sept. 7 (Sept. 12)	June 29 – (Sept.
1984				
Rate	5.14 ± 0.07	5.22 ± 0.82	7.17 ± 0.96	5.87 ±
r^2		0.93*	0.95*	0.93*
Period	Apr. 17 – Oct. 29	May 7 – July 3 (July 9)	Apr. 17 – June 4 (May 31)	May 22 – (July
Mean rate ± SEM		24.6 ± 5.6	5.5 ± 1.5	2.9 ±

[a] Slope of the linear regression of time versus the total hypolimnetic content of the chemical spe
[b] Coefficient of determination of the regression and its level of significance ($^{**}p < 0.01$; $^{*}p <$
[c] Dates included in the regression. Where applicable, the date of complete disappearance of the
[d] Only two data points available.

and increase in CH_4 and NH_4^+ during summer stratification in the hypolimnion of lake St. George in

NO_2^-	N_2O-N	$NO_3^- + NO_2^-$	CH_4	NH_4^+
2.24 ± 1.05 0.89** uly 1 – Aug. 20 (Aug. 23)	0.68 —[d] Aug. 5 – Aug. 20 (Aug. 21)	11.62 ± 0.92 0.96** July 1 – Aug. 20 (Aug. 16)	4.95 ± 0.75 0.88** May 27 – Oct. 9	3.45 ± 0.49 0.75** May 13 – Oct. 7
0.67 ± 0.45 0.54 n.s. une 1 – July 13 (July 27)	0.8 —[d] June 24 – July 21 (July 24)	3.68 ± 0.21 0.98** Apr. 22 – July 13 (July 14)	Insufficient data	3.27 ± 0.27 0.93** Apr. 22 – Oct. 5
3.13 ± 0.23 0.97** ne 14 – Aug. 16 (Aug. 13)	0.62 ± 0.13 0.96 n.s. Aug. 9 – Sept. 13 (Sept. 11)	4.53 ± 0.23 0.97** May 3 – Aug. 16 (Aug. 12)	14.83 ± 1.27 0.94** May 17 – Nov. 1	2.59 ± 0.17 0.94** Apr. 26 – Oct. 25
2.82 ± 0.51 0.94* ne 29 – Sept. 7 (Sept. 7)	0.34 —[d] Aug. 15 – Sept. 27 (Sept. 27)	5.46 ± 1.28 0.95 n.s. July 18 – Sept. 7 (Sept. 7)	8.21 ± 0.86 0.95** May 10 – Oct. 25	3.83 ± 0.57 0.90** May 18 – Sept. 27
5.87 ± 0.92 0.93* ay 22 – July 9 (July 11)	1.85 ± 0.53 0.86 n.s. July 13 – Aug. 15 (Aug. 12)	5.73 ± 0.31 0.98** Apr. 17 – July 9 (July 12)	6.27 ± 0.57 0.93** Apr. 17 – Nov. 1	1.55 ± 0.56 0.49* May 7 – July 3
2.9 ± 0.8	0.9 ± 0.3	6.2 ± 1.4	8.6 ± 2.2	2.9 ± 0.4

ical species (data from Fig. 1). Data are ± SE of the slope (Sokal and Rohlf 1981).
; $*p < 0.05$; n.s. = not significant).
e of the chemical species as obtained from the regression equation is given in parentheses.

TABLE 2. Measured change (Δ, $mmol \cdot m^{-2}$) in the hypolimnetic content of O_2, NO_3^-, and CH_4 and estimated vertical flux (VF, $mmol \cdot m^{-2}$) of these chemical species across the upper boundary of the hypolimnion during thermal stratification of Lake St. Geroge in the years 1980–84.

		O_2	NO_3^-	CH_4
1980	Δ[a]	−992	−418	641
	VF[b]	4.5 – 72.7	0.2 – 3.4	0.6 – 9.2
1981	Δ	−978	−262	Insufficient data
	VF	1.7 – 27.1	0.1 – 2.3	2.4 – 37.9
1982	Δ	−895	−403	2554
	VF	2.7 – 42.6	0.3 – 4.6	2.9 – 46.6
1983	Δ	−1560	−237	1262
	VF	2.6 – 41.9	0.3 – 4.1	1.2 – 20.0
1984	Δ	−368	−342	1819
	VF	4.4 – 70.2	0.6 – 10.1	4.1 – 65.3
Mean ± SEM	Δ	−959 ± 189	−332 ± 36	1569 ± 407
	VF	3.2 ± 0.5 – 50.9 ± 8.8	0.3 ± 0.1 – 4.9 ± 1.4	2.2 ± 0.6 – 35.8 ± 9.9

[a] Negative Δ values indicate decreases. Δ values were based on the maximum hypolimnetic content observed for each species during thermal stratification.

[b] Flux ranges were calculated as explained in Methods. The direction of flux was into the hypolimnion for O_2 and NO_3^- and out of the hypolimnion for CH_4.

significance of the regression which yielded the rate (Table 1). Finally, based on other lake studies (e.g. Kelly et al. 1982; Brock 1985), it is likely that other anaerobic processes (e.g. sulfate reduction) contribute to overall organic C mineralization in the hypolimnion of Lake St. George. These processes remain to be quantified.

Discussion

Hypolimnetic O_2 consumption during thermal stratification has long been a subject of investigation (Hutchinson 1957) and is now known to be mainly a function of surface water productivity, temperature, and lake morphometry, specifically hypolimnion thickness (Cornett and Rigler 1979; Charlton 1980; Cornett and Rigler 1980; Cornett 1989). It has also been found that total vertical transport of O_2 across the thermocline into the hypolimnion is unlikely to be greater than 15% of total hypolimnetic O_2 utilization as measured by changes in concentrations within the hypolimnion (Cornett and Rigler 1987a). Data from Lake St. George agree with this. Furthermore, photosynthetic O_2 release would not have contributed significantly to the O_2 budget of the lake's hypolimnion, since the mean depth of the euphotic zone during the five summers surveyed varied between 3.5 and 4.2 m (calculated from unpublished data provided by D. J. McQueen, York University, Toronto, Ont., and assuming that the depth of the euphotic zone is equal to 2.4 times the maximum Secchi depth; Smith 1979).

Cornett and Rigler (1987a) deplored the paucity of studies on the relative importance of sediment and water column respiration to total hypolimnetic O_2 consumption. Reported percentages of O_2 consumption occurring in the water column vary between 0 and 100% (see summary table in Cornett and Rigler 1987b). Although this percentage is likely to be strongly influenced by the average depth of the hypolimnion (Charlton 1980; Cornett and Rigler 1987b), there is, at present, no way to quantitatively predict how hypolimnetic O_2 consumption will be distributed between the sediments and the water column. However, the fact that O_2 concentrations decreased more rapidly at greater depth in the hypolimnion of Lake St. George (Knowles et al. 1990) clearly suggests that the sediments are a major site of O_2 utilisation.

There are three main pathways of NO_3^- metabolism in natural systems: assimilatory reduction, dissimilatory reduction to NH_4^+, and denitrification (Knowles 1982; Tiedje 1988). The major pathway of NO_3^- disappearance in the hypolimnion of Lake St. George during thermal stratification is assumed to be denitrification. Evidence for this includes the sequence of appearance and utilization of NO_2^- and N_2O, both of which are intermediates in denitrification, and the lack of an increase in NH_4^+ accumulation rates during NO_3^+ depletion. An earlier

TABLE 3. Estimates of organic C mineralized ($mmol\ C \cdot m^{-2}$) by the processes of O_2 and NO_3^- consumption and CH_4 and NH_4^+ production in the hypolimnion during summer stratification for Lake St. George in the years 1980–84.

	O_2[a]	NO_3^-[b]	CH_4[c]	$O_2 + NO_3^- + CH_4$	NH_4^+[d]
1980	764	523	1282	2569	6278 (10.1)
1981	753	328	—	—	5951 (10.1)
1982	689	504	5108	6301	4289 (9.2)
1983	1201	296	2524	4021	7516 (10.9)
1984	283	428	3638	4349	2812 (10.1)
Mean ± SEM	738 ± 146	416 ± 45	3138 ± 814	4310 ± 768	5369 ± 821

[a] Calculated from Δ values in Table 2 and assuming that for aerobic respiration (moles CH_2O mineralized)/(moles O_2 consumed) = 0.77 (Jørgensen 1983).

[b] Calculated from Δ values in Table 2 and assuming that for denitrification (moles CH_2O mineralized)/(moles NO_3^- consumed) = 1.25 (Brezonik 1977; Hutchinson 1957; Jørgensen 1983; Painter 1970; Wetzel 1983).

[c] Calculated from Δ values in Table 2 and assuming that for methanogenesis (moles CH_2O mineralized)/(moles CH_4 produced) = 2.00 (Brezonik 1977; Kelly and Chynoweth 1979; Wolin and Miller 1987).

[d] Based on the average daily rates of Table 1 and assuming a 180-d accumulation period. C mineralized values were obtained by applying a mean whole-lake C:N ratio (in parentheses) derived from measurements of particulate organic C and total particulate N obtained in the summers of 1982 and 1983 (Knowles et al. 1990). The C:N value for 1980, 1981, and 1984 is a mean of the 1982 and 1983 values.

study of Lake St. George sediments (Knowles 1979) provided additional evidence for the predominance of the denitrification pathway. In this study, it was found that from 59 to 100% of NO_3^- added to sediment samples obtained from 5 and 14 m was reduced to N_2O in the presence of acetylene which blocks the reduction of N_2O to N_2, the last step of denitrification (Balderston et al. 1976; Yoshinari and Knowles 1976).

The shape of the depth profile of hypolimnetic NO_3^- concentrations in Lake St. George (Knowles et al. 1990) suggests that for NO_3^- respiration, as with O_2 respiration, the sediments are a major active site. As far as can be determined, there has been no attempt to directly measure and compare water column and sediment denitrification in an anaerobic hypolimnion. If all the hypolimnetic NO_3^- consumption in Lake St. George is assumed to occur at the sediment and is due to denitrification, it can be calculated that, over the five summers studied, denitrification averaged 6.2 $mmol \cdot m^{-2} \cdot d^{-1}$ (range 3.7–11.6 $mmol \cdot m^{-2} \cdot d^{-1}$). Although this value may somewhat overestimate actual rates, it is still interesting to compare it with literature values. Denitrification rates reported from a variety of lake sediments range between 0.05 and 9.2 $mmol \cdot m^{-2} \cdot d^{-1}$, with most values falling below 1.6 $mmol \cdot m^{-2} \cdot d^{-1}$ (Seitzinger 1988), suggesting that conditions in the hypolimnion of Lake St. George are particularly favorable for NO_3^- dissimilation to N_2.

In spite of these favorable conditions, there is evidence that hypolimnetic denitrification in the lake is subject to different types of limitation. The presence of O_2 during the early part of the period of stratification may explain some of the observed NO_2^- accumulation. The later reductases of denitrifiers are known to be more sensitive to O_2 than is NO_3^- reductase (Betlach and Tiedje 1981). However, the large variability in the time of onset and rate of NO_3^- disappearance cannot be adequately explained by available data. This variability contrasts markedly with the relative constancy in the O_2 consumption pattern in years when turnover of the water column occurred.

Peaks of N_2O, seen in August during 1982 and 1983, long after O_2 had been completely consumed, may be due to H_2S inhibition of N_2O reductase (Tam and Knowles 1979; Sørensen et al. 1980). H_2S is clearly detectable by smell in hypolimnetic waters (C. Bédard, unpubl. data).

The denitrification rate in hypolimnetic sediments calculated in the present study is some 30 times greater than the rate in epilimnetic sediments as determined during summer by acetylene blockage (Chan and Knowles 1979). Lower epilimnetic denitrification rates may be related to competition with the assimilatory pathway of NO_3^- reduction and the overall availability of NO_3^-. It has been pointed out that, in most lakes, epilimnetic sediment area is greater than the hypolimnetic area (as is the case in Lake St. George) and therefore the contribution of the epilimnion tends to be more important on a whole-lake basis (Kelly et al. 1982). In Lake St. George, it can be calculated that total denitrification in epi- and metalimnetic sediments over a 150-d stratification period will be 1.1 kmol of N if an average daily rate of 0.2 $mmol \cdot m^{-2}$ (Chan and Knowles 1979) is assumed. Over the five summers for which data are available, the hypolimnetic sediments denitrified, on average, 3.5 kmol of N. Extrapolation of whole-lake denitrification values from measurements obtained in a limited number of locations at a single time can be misleading (Messer and Brezonik 1983). However, the above calculations for Lake St. George at least suggest that total denitrification in the hypolimnion during the period of thermal stratification is within the same order of magnitude as in the epilimnion over the same period.

CH_4 production in lakes is essentially limited to the sediments (Zeikus and Winfrey 1976; Rudd and Hamilton 1978). Therefore, CH_4 production values for the sediments of Lake St. George can be readily derived: in the four summers for which adequate data are available, rates ranged between 5.0 and 16.6 mmol $CH_4 \cdot m^{-2} \cdot d^{-1}$, averaging 8.6 $mmol \cdot m^{-2} \cdot d^{-1}$. There has been a number of studies of CH_4 production in anaerobic hypolimnetic sediments during the period of thermal stratification (Strayer and Tiedje 1978; Rudd and Hamilton 1978; Kelly and Chynoweth 1979; Fallon et al. 1980; Kelly et al. 1982). Average seasonal production rates in these studies ranged between 3.5 and 36 mmol $CH_4 \cdot m^{-2} \cdot d^{-1}$. The apparent predominance of methanogenesis as a pathway of organic C mineralization in the hypolimnion has been observed in other lakes with an anoxic hypolimnion (Kelly and Chynoweth 1981; Kelly et al. 1982; Brock 1985).

On average, approximately 20% of organic C mineralization in the hypolimnion of Lake St. George as estimated from NH_4^+ accumulation could not be accounted for by O_2 respiration, denitrification, or methanogenesis. A process which was not measured, but is likely to be important, is sulfate reduction. For example, sulfate reduction was responsible for 16–20% of total organic C mineralization in the hypolimnion of three Canadian Shield Lakes during thermal stratification (Kelly et al. 1982, 1988). The presence of H_2S in the hypolimnion of Lake St. George and the occurrence of large populations of photosynthetic sulfur-bacteria (R. Knowles and C. Bédard, unpubl. data) are evidence for the importance of hypolimnetic sulfur cycling. Reduction of iron and manganese, also not determined here, is not likely to be very important (Kelly et al. 1988).

Annual primary productivity in Lake St. George has been estimated to amount to 875 kmol of C based on ^{14}C fixation (M. N. Charlton and D. R. S. Lean, National Water Research Institute, Burlington, Ont. L7R 4A6, unpubl. data). Estimates of average organic C mineralization in the hypolimnion during thermal stratification account for only 6% of this value.

The factors affecting aerobic mineralization rates in the hypolimnion, no doubt, can also control anaerobic mineralization rates. For example, methanogenesis in anaerobic hypolimnia appears to be directly related to temperature and organic C input (Kelly and Chynoweth 1981). It may be possible, therefore, to expand present models which predict rates of hypolimnetic O_2 utilization (Cornett and Rigler 1979, 1990; Charlton 1980; Cornett 1989) to include anaerobic processes. Further challenges will be to predict the rates of individual processes, such as denitrification and sulfate reduction, and their distribution between sediments and the water column.

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References

BALDERSTON, W. L., B. SHERR, AND W. J. PAYNE. 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. Environ. Microbiol. 31: 504–508.

BETLACH, M. R., AND J. M. TIEDJE. 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. 42: 1072–1084.

Brezonik, P. L. 1977. Denitrification in natural waters. Prog. Water Technol. 8: 373–392.

Brock, T. D. 1985. A eutrophic lake. Lake Mendota, Wisconsin. Springer-Verlag, New York, NY. 307 p.

Chan, Y.-K., and R. Knowles. 1979. Measurement of denitrification in two freshwater sediments by an in situ acetylene inhibition method. Appl. Environ. Microbiol. 37: 1067–1072.

Charlton, M. N. 1980. Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects. Can. J. Fish. Aquat. Sci. 37: 1531–1539.

Cornett, R. J. 1989. Predicting changes in hypolimnetic oxygen concentrations with phosphorus retention, temperature, and morphometry. Limnol. Oceanogr. 34: 1359–1366.

Cornett, R. J., and F. H. Rigler. 1979. Hypolimnetic oxygen deficits: their prediction and interpretation. Science (Wash., DC) 205: 580–581.

1980. The areal hypolimnetic oxygen deficit: an empirical test of the model. Limnol. Oceanogr. 25: 672–679.

1987a. Vertical transport of oxygen into the hypolimnion of lakes. Can. J. Fish. Aquat. Sci. 44: 852–858.

1987b. Decomposition of seston in the hypolimnion. Can. J. Fish. Aquat. Sci. 44: 146–151.

Fallon, R. D., S. Harrits, R. S. Hanson, and T. D. Brock. 1980. The role of methane in internal carbon cycling in Lake Mendota during summer stratification. Limnol. Oceanogr. 25: 357–360.

Hutchinson, G. E. 1957. A treatise on limnology. I. Geography, physics and chemistry. John Wiley and Sons, New York, NY. 1015 p.

Jørgensen, B. B. 1983. Processes at the sediment-water interface, p. 477–515. *In* B. Bolin and R. B. Cook [ed.] The major biogeochemical cycles and their interactions. John Wiley & Sons, Chichester.

Kelly, C. A., and D. P. Chynoweth. 1979. Methanogenesis: a measure of chemoorganotrophic (heterotrophic) activity in anaerobic lake sediments, p. 164–179. *In* R. R. Colwell and W. Costerton [ed.] Native aquatic bacteria, enumeration, activity and ecology. Proceedings of the American Society for Testing and Materials Symposium, Minneapolis, June 1977. American Society for Testing and Materials, Philadelphia, PA.

1980. Comparison of in situ and in vitro rates of methane release in freshwater sediments. Appl. Environ. Microbiol. 40: 287–293.

1981. The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. Limnol. Oceanogr. 26: 891–897.

Kelly, C. A., J. W. M. Rudd, R. B. Cook, and D. W. Schindler. 1982. The potential importance of bacterial processes in regulating rate of lake acidification. Limnol. Oceanogr. 27: 868–882.

Kelly, C. A., J. W. M. Rudd, and D. W. Schindler. 1988. Carbon and electron flow via methanogenesis, SO_4^{2-}, NO_3^-, Fe^{3+}, and Mn^{4+} reduction in the anoxic hypolimnia of three lakes. Arch. Hydrobiol. Beih. Ergebn. Limnol. 31: 333–344.

Knowles, R. 1979. Denitrification, acetylene reduction, and methane metabolism in lake sediment exposed to acetylene. Appl. Environ. Microbiol. 38: 486–493.

1982. Denitrification. Microbiol. Rev. 46: 43–70.

Knowles, R., C. Bedard, D. R. S. Lean, and D. J. McQueen. 1990. Lake St. George, Ontario, 1976 to 1984: seasonal changes in temperature, oxygen, chlorophyll, methane and several nitrogen fractions. Natl. Water Res. Inst. (Burlington) Publ. No. 89-42: 36 p and appendices (50 p.).

Knowles, R., and D. R. S. Lean. 1987. Nitrification: a significant cause of oxygen depletion under winter ice. Can. J. Fish. Aquat. Sci. 44: 743–749.

Knowles, R., D. R. S. Lean, and Y.-K. Chan. 1981. Nitrous oxide concentrations in lakes: variations with depth and time. Limnol. Oceanogr. 26: 855–866.

Messer, J., and P. L. Brezonik. 1983. Comparison of denitrification rate estimation techniques in a large, shallow lake. Water Res. 17: 631–640.

Painter, H. A. 1970. A review of literature on inorganic nitrogen metabolism in microorganisms. Water Res. 4: 393–450.

Quay, P. D., W. S. Broecker, R. H. Hesslein, and D. W. Schindler. 1980. Vertical diffusion rates determined by tritium tracer experiments in the thermocline and hypolimnion of two lakes. Limnol. Oceanogr. 23: 337–348.

Rudd, J. W. M., and R. D. Hamilton. 1978. Methane cycling in a eutrophic shield lake and its effects on whole lake metabolism. Limnol. Oceanogr. 23: 337–348.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceanogr. 33: 702–724.

Smith, V. H. 1979. Nutrient dependence of primary productivity in lakes. Limnol. Oceanogr. 24: 1051–1064.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co., San Francisco, CA. 859 p.

Sørensen, J., J. M. Tiedje, and R. B. Firestone. 1980. Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 39: 105–108.

Strayer, R. F., and J. M. Tiedje. 1978. In situ methane production in a small, hypereutrophic, hard-water lake: loss of methane from sediments by vertical diffusion and ebullition. Limnol. Oceanogr. 23: 1201–1206.

Tam, T.-Y., and R. Knowles. 1979. Effects of sulfide and acetylene on nitrous oxide reduction by soil and by *Pseudomonas aeruginosa*. Can. J. Microbiol. 25: 1133–1138.

Tiedje, J. M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium, p. 179–243. *In* A. J. B. Zehnder [ed.] Biology of anaerobic microorganisms. John Wiley and Sons, New York, NY.

Wetzel, R. G. 1983. Limnology. 2nd ed. Saunders College Publishing, Philadelphia, PA. 767 p.

Wolin, M. J., and T. L. Miller. 1987. Bioconversion of organic carbon to CH_4 and CO_2. Geomicrobiol. J. 5: 239–259.

Yoshinari, T., and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Commun. 69: 705–710.

Zeikus, J. G., and M. R. Winfrey. 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl. Environ. Microbiol. 31: 99–107.

Relationship between Chronic Toxicity and Bioaccumulation of Cadmium in *Hyalella azteca*

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Borgmann, U., W. P. Norwood, and I. M. Babirad. 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 48: 1055–1060.

The chronic toxicity of Cd to *Hyalella* in Burlington City tap (Lake Ontario) water with additions of complexing agents, distilled water, or sediments was much more constant if toxicity was expressed as a function of Cd bioaccumulated, rather than the Cd concentrations added or measured in the water. Additions of 20 mg humic acid/L or 0.5 μM EDTA increased the 6-wk EC50 from 0.53 to 4.6 and 19 μg/L, respectively. The EC50 based on bioaccumulated Cd, however, increased from 38 to only 44 μg/g (dry weight). Addition of sediments increased the 4-wk EC50 by >1000-, 13-, or 2.3-fold for EC50s based on nominal Cd added, Cd concentrations measured in water, or Cd concentrations accumulated by *Hyalella*, respectively. A 10-fold reduction in hardness caused a twofold increase in the 6-wk EC50 based on Cd accumulation. The relationship between survival and Cd bioaccumulation by *Hyalella* was, therefore, affected less by complexing agents, hardness, and sediments than was survival compared with concentrations in water. Published Cd concentrations in *Hyalella* from a number of Ontario lakes are close to levels associated with toxicity in the laboratory.

La toxicité chronique du Cd présent dans l'eau courante de Burlington, tirée du lac Ontario, a été établie dans le cas de *Hyalella*. Avec des ajouts d'agents chélatants, d'eau distillée ou de sédiments, la toxicité chronique était nettement plus constante si elle était exprimée comme fonction du Cd accumulé au lieu des concentrations de Cd ajoutées ou déterminées dans l'eau. L'ajout de 20 mg d'acide humique/L ou de 0,5 μM d'EDTA a fait passer la CE50 après 6 sem de 0,53 à 4,6 et à 19 μg/L, respectivement. Toutefois, la CE50 basée sur la teneur en Cd bioaccumulé n'a que légèrement augmenté; elle est passée de 38 à 44 μg/g (poids sec). L'ajout de sédiments a entraîné une augmentation de la CE50 après 4 sem par plus de 1 000, par 13 et par 2,3 fois dans le cas des CE50 basées sur la teneur nominale en Cd ajoutée, les teneurs en Cd de l'eau et les teneurs en Cd bioaccumulées par *Hyalella*, respectivement. Une baisse de la dureté par 10 a entraîné une augmentation par deux de la CE50 après 6 sem basée sur la teneur en Cd accumulée. La relation entre le taux de survie de *Hyalella* et la bioaccumulation de Cd est donc moins touchée par les agents chélatants, la dureté de l'eau et les sédiments que la relation entre le taux de survie et les teneurs en Cd de l'eau. Les teneurs en Cd relevées chez *Hyalella* dans d'autres lacs de l'Ontario se rapprochent des teneurs toxiques en laboratoire.

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Most laboratory toxicity studies have examined only the relationship between toxicity and toxicant concentrations in water, and most water quality criteria or objectives are based on such studies. For example, the Water Quality Objective for Cd in the Great Lakes is 0.2 μg/L (Canada–U.S. Water Quality Agreement) and is based, in part, on the study by Biesinger and Christensen (1972) which showed reproductive impairment in *Daphnia magna* at 0.17 μg/L (International Joint Commission 1976). This was the most sensitive organism reported in the literature at the time the objective was written. The amphipod *Hyalella azteca* has recently been shown to be as sensitive as *D. magna* to Cd in water (Borgmann et al. 1989). However, the sensitivity of aquatic organisms to metals in water is dependent on the chemical characteristics of the water. Hardness, pH, chemical complexing agents and particulate matter all influence toxicity (Borgmann 1983). This makes it difficult to infer actual toxicity in the field from only measurements of metal concentrations in water.

In contrast, the metal concentrations in the organism, rather than in the water, have usually been the focus of field studies. Stephenson and Mackie (1988), for example, measured Cd levels in *H. azteca* in 69 central Ontario lakes. Although *Hyalella* is a benthic organism, Cd levels in the amphipods did not correlate with Cd levels in the sediments, but did correlate with Ca, dissolved organic carbon (DOC), and Cd levels in water. Unfortunately, direct comparisons between such studies, based on metal bioaccumulated, and laboratory toxicity studies, based on concentrations in water, cannot be made. Several authors (e.g. Davies 1978; Connolly 1985) have suggested that toxicity should correlate more closely with metal concentrations in organisms than concentrations in the water and that measurement of tissue concentrations would make laboratory studies much more useful in evaluating the effects of metals in the field. In the present study, we compare chronic toxicity with Cd bioaccumulation by *Hyalella* in water of varying hardness and chemical complexing capacity and in the presence and absence of sediments.

Methods

Amphipods were cultured as described in Borgmann et al. (1989). Toxicity tests without sediment were initiated with twenty 0- to 1-wk-old young in 250 mL of dechlorinated Burlington City tap water (originating from Lake Ontario) in

500-mL Erlenmeyer flasks with loose-fitting glass covers. Each flask contained one 5 × 10 cm piece of presoaked cotton gauze as substrate. Water and Cd were changed once a week, at which time the number of survivors were counted, young were counted and removed, and 5 mg of Tetra-Min fish food flakes was added as food. Additional food was added up to two times per week, if required. The animals were weighed on week 6, when the experiment was terminated. Six weeks is long enough to determine chronic Cd toxicity to *Hyalella* under our conditions (Borgmann et al. 1989). All experiments were conducted in an incubator at 25°C with a 16 h light : 8 h dark photoperiod, using fluorescent bulbs placed 35–40 cm above the shelves. Four replicates were run for each treatment: two were set up one week and two another week. Experiments were conducted in dechlorinated tap water (hardness 130 mg/L, alkalinity 90 mg/L, Ca 40 mg/L, Mg 8 mg/L, Na 12 mg/L, SO_4^{2-} 28 mg/L, Cl^- 24 mg/L, and DOC 2 mg/L) originating from Lake Ontario, tap water with 0.5 μM ethylenediaminetetraacetic acid (EDTA) or 20 mg humic acid/L (Aldrich Chem. Co.), or 10% tap water plus 90% glass-distilled water. One experiment with only two replicates was conducted in the presence of 1 μM 8-hydroxyquinoline (8HQ). It was not repeated because survival did not appear to be affected by this ligand. The initial pH ranged from 7.9 to 8.7 in all experiments except 90% distilled water, which ranged from 7.1 to 7.4. The final pH after each week of incubation occasionally reached 9 due to algal photosynthesis.

Cadmium solutions were prepared from a Cd metal standard dissolved in nitric acid and added to the food and water in each flask. The flasks were allowed to sit for several hours before the animals were added to ensure that any partitioning between food, glass, and metal in water had come to equilibrium. After each water change, water samples were preserved with nitric acid for metal analysis. Cadmium in water was measured by the National Water Quality Laboratory, Inland Waters Directorate, Department of Environment, using APDC/MIBK solvent extraction followed by flame atomic absorption spectrophotometry on a Perkin-Elmer 5000 or 5100 atomic absorption spectrophotometer with deuterium lamp background correction. The QA/QC protocol included running samples in duplicate, spike and recovery measurements, in house controls, and NBS and other certified standards.

At the end of the experiments, one half of the amphipods were placed over nylon mesh screens in clean water to clear their guts before drying; the other half were analyzed directly. Although Cd concentrations in *Hyalella* were measured only at the end of the experiments, a preliminary study suggested that Cd concentrations were equilibrated within 1 wk. The amphipods were dried at 60°C, weighed in polypropylene pans on a microbalance (±0.1 mg), and placed in polypropylene microcentrifuge tubes for digestion. The amphipods were digested following the procedure of Stephenson and Mackie (1988). Twenty-five microlitres of 70% nitric acid was added to one to four amphipods (0.5–2 mg dry weight) and allowed to sit for 1 wk. Then, 16.6 μL of 30% hydrogen peroxide (equivalent to 10 μL of 50% hydrogen peroxide; Stephenson and Mackie 1988) was added and the samples were allowed to sit overnight. One millilitre of distilled water was then added. Microscopic analysis revealed that no tissue fragments remained and the solutions were clear. Tissue digests were analyzed for Cd by D. Kurylo, Great Lakes Forestry Centre, Department of Forestry, Sault Ste. Marie, using a Perkin-Elmer 4000 graphite furnace atomic absorption spectrophotometer with deuterium lamp background correction and EPA reference samples. Some of the same samples were later reanalyzed on a Varian SpectrAA 400 graphite furnace atomic absorption spectrophotometer with Zeeman background correction and were found to contain the same amount of Cd as previously measured. Digestion of an NRC TORT-1 lobster certified (26.3 ± 2.1 μg Cd/g) sample gave a measured Cd concentration of 25.2 μg/g. Since analysis of variance indicated that there was no difference between Cd in amphipods after 0 and 24 h of gut clearance, the 0- and 24-h data were pooled.

Experiments with sediments were conducted by adding 20 mL of Hamilton Harbour site 1 sediment (Borgmann and Munawar 1989) to 180 mL of water in 250-mL beakers, adding Cd, and aerating vigorously for 24 h. The mixture was allowed to settle and then aerated gently before 20 young amphipods were added. Gentle aeration was provided through a glass pipette to ensure adequate oxygen levels for the duration of the sediment tests. Five milligrams of fish food flakes were added three times per week per beaker. Unlike in the tests without sediment, water and Cd were not changed weekly, but were left until the end of the experiment. These experiments were terminated after 4 wk, a recommended time for sediment toxicity tests (Borgmann and Munawar 1989). Two replicates were run at each Cd concentration. The experiment was performed twice, once using sediments collected in May and once using sediments collected in August 1989. Metal concentrations in the decanted water and in the amphipods were measured as in the experiments without sediment, except that all amphipods were placed over a nylon screen in clean water overnight to clear their guts and to provide extra time for any adhering sediment particles to dislodge. The sediments were dried at 60°C, ground, digested in 0.5 N HCl, and analyzed for Cd by the National Water Quality Laboratory using flame atomic absorption spectrophotometry on a Perkin-Elmer 4000 atomic absorption spectrophotometer with deuterium lamp background correction and a QA/QC protocol similar to the water samples.

The concentrations resulting in survival equal to 50% of control survival (the EC50) and their 95% confidence limits were computed from regressions of survival against log concentration in the linear portion of the survival–concentration curve (the three or four concentrations near the EC50) using survival and bioaccumulation data for each replicate (four replicates per concentration for the experiments without sediments and two for each experiment with sediment).

Results

For the experiments without sediments, the concentration of Cd in the water 1 wk after Cd addition was proportional to the amount of Cd added. At nominal Cd concentrations ≥1.0 μg/L, each measured concentration was significantly lower than the next higher concentration (analysis of variance after log transformation, $P < 0.05$). However, the final concentration was a function of hardness and complexing capacity (Table 1). Cadmium recovery was higher in the 90% distilled water and in the presence of complexing agents. Cadmium concentrations immediately after Cd addition were not measured in this study. Subsequent experiments suggest that the "loss" of Cd is due to adsorption onto the glass, gauze, and/or particulate matter. This appears to occur within a few hours after Cd addition, and the "lost" Cd can be recovered by acidifying the entire flask and its contents, instead of decanting the water prior to acidification. Very high Cd additions were required to reduce survival in the presence of sediments, and little of this Cd remained

TABLE 1. Measured Cd concentrations in water (µg/L ± SD) at the end of each 1 wk of exposure for the various treatments without sediments and the overall mean percent recovery of Cd.

Nominal Cd (µg/L)	Treatment			
	Tap water	Humic acid	EDTA	90% distilled
Control (0)	<0.18	<0.10	<0.10	<0.10
0.18	—	—	—	<0.16
0.32	<0.15	—	—	<0.18
0.56	<0.18	—	—	0.38 ± 0.13
1.0	0.34 ± 0.11	0.55 ± 0.16	—	0.54 ± 0.09
1.8	0.56 ± 0.13	1.05 ± 0.29	—	1.06 ± 0.19
3.2	0.99 ± 0.25	2.28 ± 0.33	—	2.20 ± 0.17
5.6	1.77 ± 0.46	3.68 ± 0.43	4.84 ± 0.26	—
10	3.1	6.15 ± 1.33	8.98 ± 0.83	—
18	—	11.0 ± 1.0	15.3 ± 0.9	—
32	—	—	27.4 ± 1.6	—
56	—	—	44.5 ± 6.1	—
Mean % recovery	31	63	84	62

TABLE 2. Final measured Cd concentrations in water (µg/L) and sediment (µg/g dry weight) in the treatments with sediments and the overall mean percent recovery of Cd. Recovery refers to Cd recovered from the sediments alone, based on a 1:9 sediment to water ratio and a sediment water content of 69% in experiment A and 76% in experiment B.

Nominal Cd (µg/L)	Sediment A		Sediment B	
	µg/L	µg/g	µg/L	µg/g
Control (0)	—	4.67	0.8	—
1 000	3.6	29.0	—	—
1 800	5.8	49.7	3.5	63.0
3 200	10.9	83.0	8.0	113
5 600	21.2	141	10.5	196
10 000	—	—	6.7	350
18 000	—	—	8.2	648
32 000	—	—	24.5	—
Mean % recovery		83		85

in the water. Cadmium concentrations in sediments were proportional to the amount of Cd added and accounted for 84% of the total Cd added. Concentrations in the overlying water tended to increase with increasing Cd added, but not in a smooth fashion (Table 2).

Survival after 4–6 wk was strongly affected by both complexing agents and sediments (Fig. 1). The nominal 6-wk EC50 (concentration resulting in survival equal to 50% of control survival; control survival is listed in Table 5) was increased from 1.6 in tap water to 7.0 µg/L after addition of humic acid and to 22 µg/L after addition of EDTA (Table 3). Between 2000 and 6300 µg/L of added Cd was required to cause the same mortality in the presence of sediments, although only about 7 µg/L remained in the water by the end of the experiments. Hardness, on the other hand, had only a minor effect on Cd toxicity. Mortality was slightly higher in 90% distilled water based on nominal Cd concentrations, but slightly lower based on final measured concentrations (Table 3).

The Cd bioaccumulation curves in the presence of humic acid and EDTA were shifted to the right in the same manner as the survival curves (Fig. 2). Cadmium bioaccumulation was slightly higher in the presence of sediments. Hardness had only a minor effect on bioaccumulation, but final body concentrations were higher in the 90% distilled water, especially at the higher and more toxic concentrations. The slopes (±95% confidence limits) of the regression of log Cd bioaccumulation against log Cd measured are 0.52 ± 0.12, 0.47 ± 0.09, 0.85 ± 0.14, and 0.97 ± 0.11 for tap water, humic acid, EDTA, and 90% distilled water, respectively. A strong complexing agent, such as EDTA, which prevents bioaccumulation until the complexing capacity of the EDTA is exceeded, would be expected to increase the slope. The reason for the higher slope in the 90% distilled water as compared with the tap water is not known. Slopes of 0.64–0.83 have been reported for the relationship between log Cd in *Hyalella* from the field and log

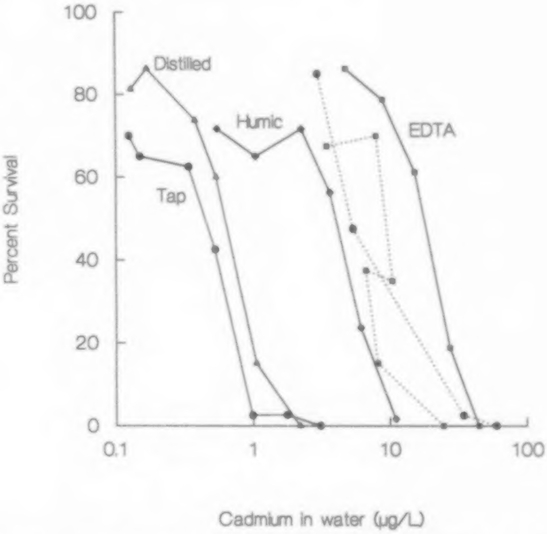

FIG. 1. Percent survival after 4 wk (sediment experiments) or 6 wk (all experiments without sediments) as a function of measured concentrations of Cd in tap water (●, Tap), 20 mg humic acid/L (◆, Humic), 0.5 µM EDTA (■, EDTA), 90% distilled water (▲, Distilled), or 10% sediment by volume (broken lines: ●, experiment A; ■, experiment B).

TABLE 3. Concentration resulting in 50% survival (EC50) based on nominal Cd concentration added, measured Cd in the water, and measured concentration in the amphipods. Exposure duration is 6 wk in the absence of sediments and 4 wk with sediment. Numbers in parentheses are 95% confidence limits.

	EC50		
Treatment	Nominal (μg/L)	Measured (μg/L)	Bio-accumulated (μg/g)
Tap water	1.6 (0.9–2.8)	0.53 (0.33–0.84)	38
Humic acid	7.0 (2.9–17)	4.6 (2.0–10)	44
EDTA	22 (13–37)	19 (12–30)	44
90% distilled	1.2 (0.6–2.6)	0.72 (0.45–1.2)	79
Sediment A	2000 (900–4900)	6.8 (3.1–16)	86
Sediment B	6300 (1700–24 000)	7.4 (0.7–59)	98

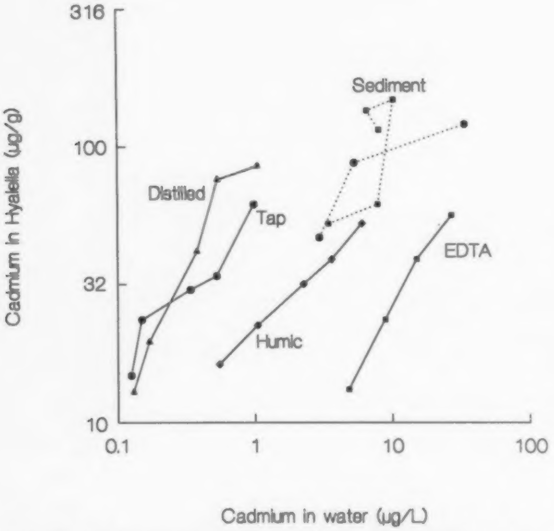

FIG. 2. Cadmium accumulation (μg/g dry weight) after 4–6 wk as a function of Cd concentrations in the water. Symbols as in Fig. 1.

Cd in water, depending on whether DOC is, or is not, included in the regression (Stephenson and Mackie 1988). These fall within the range of our slopes.

When the survival was plotted as a function of Cd accumulated, rather than Cd in water, the survival curves lay much closer to one another, but they did not overlap completely (Fig. 3). Addition of humic acid or EDTA shifted the curve slightly to the right. These two curves lay virtually on top of one another. The survival curve was shifted further to the right in 90% distilled water, and still further in the presence of sediments. Figure 3 includes data from controls (no Cd addition, lowest concentration on each line); these are not shown in Fig. 1 and 2 because most measurements of Cd in water without added Cd were below the detection limit. The EC50s, as a function of Cd accumulated, were much more uniform than EC50s based on either nominal or final measured concentrations in water (Table 3). There was a 5200-, 36-, and 2.6-fold range in the EC50s based on nominal Cd, Cd measured in water, and Cd measured in *Hyalella*, respectively. The EC50s based on bioaccumulated Cd were all not significantly different from one another.

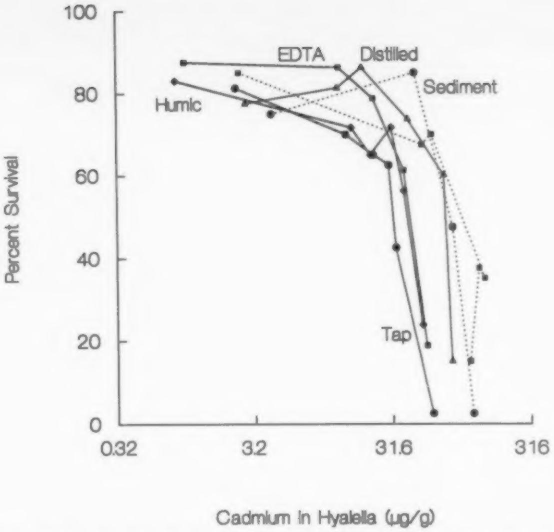

FIG. 3. Percent survival after 4–6 wk as a function of Cd accumulated. Symbols as in Fig. 1.

The Cd bioaccumulated at the EC50 can be compared with bioconcentration factors observed in the field. The relationship shown in fig. 2 of Stephenson and Mackie (1988) for *Hyalella* from central Ontario lakes gives predicted Cd accumulated values of 15, 88, and 128 μg/g for tap water, humic acid, and 90% distilled water, respectively. This compares with observed values of 38, 44, and 79 μg/g, respectively. Including DOC in the regression (table 5 of Stephenson and Mackie 1988), however, gives predicted Cd accumulated concentrations of only 4.0 and 7.0 μg/g for tap water and humic acid, respectively. The predicted Cd accumulation in 90% distilled water would be 771 μg/g if it is assumed that all the DOC is removed by distillation (final DOC = 0.2 mg/L) and 120 μg/g if distillation removes none of the DOC. The predicted concentrations, therefore, cover the range of observed bioaccumulation in the laboratory experiments, but the Ca concentration has a much smaller influence on Cd bioaccumulation in our study than in that of Stephenson and Mackie (1988). Perhaps this is partly because most of our experiments were conducted in water with a Ca concentration of 40 mg/L whereas the field study included lakes with Ca ranging from 2 to only 20 mg/L.

The highest "safe" (no significant mortality) Cd concentrations observed in amphipods in the various experiments overlap the range of concentrations found to be toxic (Table 4). This again demonstrates that toxicity cannot be perfectly described by Cd accumulated, although the range of overlapping concentrations (28–62 μg/g) is not large in comparison with the range in Cd additions. Table 4 also includes the results from a single experiment with 1 μM 8HQ. Mortality was expected to be greater in the presence of this complexing agent, and Cd concentrations of only up to 1 μg/L were tested. This chemical did not, however, reduce survival of *Hyalella*, and further tests were not conducted. Cadmium bioaccumulation was also not increased by 8HQ (Table 4).

The Cd concentrations in control amphipods (not exposed to added Cd, left-most data points in Fig. 3) were less than 10% of levels associated with mortality in any given experiment (Table 5). Concentrations in control amphipods in the presence of humic acid or EDTA were substantially lower than in the

TABLE 4. Highest concentration of Cd added at which no significant (chi-square, $P < 0.05$) mortality occurred, lowest concentration with significant mortality, and geometric mean of the Cd bioaccumulated, number of bioaccumulation measurements (n), and standard deviation of the logarithm of Cd bioaccumulated (SD) at these concentrations.

	Highest nontoxic				Lowest toxic			
		Bioaccumulated				Bioaccumulated		
Treatment	Nominal (μg/L)	μg/g	n	SD	Nominal (μg/L)	μg/g	n	SD
Tap water	0.56	23	7	0.07	1.0	30	12	0.10
Humic acid	3.2	32	10	0.08	5.6	39	10	0.06
EDTA	10	23	10	0.05	18	39	10	0.12
8HQ[a]	0.56	13	6	0.07	1.0	28	6	0.13
90% distilled	0.56	42	10	0.12	1.0	76	10	0.07
Sediment A	1000	47	4	0.09	1800	87	3	0.05
Sediment B	3200	62	4	0.07	5600	148	4	0.07

[a] One experiment only.

TABLE 5. Percent survival (%S), the geometric mean Cd bioaccumulated, and the geometric mean wet weight of *Hyalella* not exposed to added Cd. Number of measurements (n) and standard deviation of the logarithm of the Cd bioaccumulated or weight (SD) are also shown.

		Cd bioaccumulated			Size at 6 wk		
Treatment	%S	μg/g	n	SD	mg	n	SD
Tap water	81	2.36	13	0.19	1.30	16	0.12
Humic acid	83	0.84	12	0.23	2.04	19	0.14
EDTA	88	0.99	10	0.25	1.19	15	0.10
90% distilled	78	2.73	8	0.21	1.17	20	0.13
Sediment A	75	4.24	4	0.09	—	—	—
Sediment B	85	2.47	4	0.03	—	—	—

other experiments, indicating that some Cd contamination was present in all our experiments and its bioaccumulation was reduced by these complexing agents.

Cadmium had no effect on growth in any experiment when compared with controls in the same water but without added Cd (analysis of variance, $P < 0.05$). In the presence of humic acid, however, the final body size was increased significantly, both in controls (Table 5) and at all Cd concentrations. The reason for this stimulation is not known. It is probably not associated with the metal-complexing effect of humic acid because EDTA did not stimulate growth.

Discussion

Overall, Cd concentrations in the amphipods were much more uniform in treatments with equivalent survival than were concentrations in the water. Complexing agents, such as humic acid, EDTA, and sediment extracts, bind to metals in solution and reduce the amount of free metal ions available for uptake. Toxicity is often, but not always, more closely related to free metal ion concentrations than to total metal in solution (Borgmann 1983). Free metal ion concentrations are, however, not always easily measured on a routine basis. An alternative is to measure the total metal bioaccumulated and to determine the relationship between bioaccumulation and toxicity. The humic acid and EDTA concentrations used in the present study were chosen to provide a relatively high degree of complexation so that the constancy of the relationship between toxicity and bioaccumulation could be evaluated in waters with widely varying total metal concentrations.

The survival–bioaccumulation curve in the presence of the 90% distilled water was shifted slightly to the right. The EC50, based on bioaccumulation, was about twofold higher in this water than in all other waters without sediments (Table 3), although the difference is not statistically significant. It is possible that this resulted from a reduced competition between Cd and Ca ions, resulting in a greater bioaccumulation and storage of Cd in tissues which are not the site of toxic action, possibly the carapace. Calcium ions have previously been shown to inhibit Cd bioaccumulation in *Hyalella* (Stephenson and Mackie 1989a). The survival–bioaccumulation curve was also shifted to the right in the presence of sediments. Perhaps this resulted in increased adsorption onto the exoskeleton. Alternatively, ingestion of contaminated sediments might have resulted in increased bioaccumulation in the gut in tissues which, again, were not the site of toxic action. The 4- to 6-wk EC50 was approximately 2- to 2.6-fold higher in the presence of sediments than in their absence, based on concentrations in the animals. This was, however, a much smaller increase than one based on nominal or final measured concentrations in water. The 4-wk EC50 for bioaccumulated Cd in the presence of sediment was similar to the lethal threshold for juvenile *Pontoporeia affinia* (80–90 μg/g), a brackish water amphipod from the Baltic, also tested in the presence of sediment but at 2–6°C and 7 g/L salinity (Sundelin 1983).

Some metal complexes are lipid soluble, readily bioaccumulated, and extremely toxic to aquatic organisms. For example, Cu complexes of 8HQ are more toxic to algae and amphipods than Cu alone (Florence et al. 1983; Ahsanullah and Florence 1984). We tested the effect of 1 μM 8HQ on Cd toxicity to *Hyalella* in one experiment, expecting to see an increased toxicity. However, survival was virtually identical to survival without added complexing agents, so the experiment was not repeated. Although Cd toxicity was not increased by 1 μM 8HQ, neither was Cd bioaccumulation (Table 4). Cadmium bioaccumulation at concentrations below 1 μg/L was actually reduced by addition of 8HQ. Addition of 8HQ did not, therefore, help to further evaluate the relationship between Cd toxicity and bioaccumulation by *Hyalella*.

Winner and Gauss (1986) were unable to show a consistent relationship between Cd toxicity and bioaccumulation in *Daphnia*. However, they measured chronic toxicity to *D. pulex* and compared this with bioaccumulation by *D. magna*. They observed a decreased toxicity with increasing hardness, but no change in bioaccumulation, over a fourfold range in hardness. This is different from our results but must be interpreted with caution because bioaccumulation and toxicity were not measured in the same species and because Cd bioaccumulation had not equilibrated within the exposure period. Humic acid did not affect bioaccumulation of Cd by *Daphnia*, but Winner and Gauss (1986) used a humic acid concentration of only 0.75 mg/L, much lower than in our experiments.

Our results provide an approximate estimate of the tissue concentration which is likely to be associated with toxicity in natural populations. Overall, Cd concentrations in *Hyalella* of 23 μg/g or less did not result in mortality in any of our experiments (Table 4), and concentrations of Cd which do not cause mortality also do not affect growth or reproduction in *Hyalella* (Borgmann et al. 1989). Depending on the medium, concentrations of 28–62 μg/g were, or were not, associated with mortality. Concentrations of 76 μg/g and above were always toxic. These results can be compared with Cd concentrations measured in *Hyalella* from the field.

Cadmium in *Hyalella* in 69 central Ontario Lakes ranged from 0.13 to 56.5 μg/g (Stephenson and Mackie 1988). The highest value was obtained from a single amphipod from Plastic Lake; all other measurements are based on the mean of 7–10 animals. Two lakes had amphipods with Cd concentrations of 36–37 μg/g, although levels as high as 54 μg/g were recorded in one of these lakes in a more intensive study (Stephenson and Mackie 1989b). Of the 69 lakes, nine had *Hyalella* with Cd concentrations between 20 and 25 μg/g; the rest were lower. Three of the study lakes, therefore, had amphipods with Cd concentrations above those which sometimes caused mortality in our studies, and nine others had amphipods with concentrations close to our lower limit for toxicity. Since most of the lakes studied by Stephenson and Mackie (1988) had relatively low hardness, and since the *Hyalella* from these lakes would have had access to sediments, it is likely that they would tolerate Cd concentrations closer to the high end of those observed in our studies. The concentrations of 54–56 μg/g reported by Stephenson and Mackie (1988) may, therefore, be below the toxic threshold. However, Cd levels in a number of Ontario lakes appear to be close to being toxic to *Hyalella*. This conclusion might be modified if future studies demonstrate that *Hyalella* can adapt to elevated Cd concentrations after prolonged, multigeneration exposure.

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References

AHSANULLAH, M., AND T. M. FLORENCE. 1984. Toxicity of copper to the marine amphipod *Allorchestes compressa* in the presence of water- and lipid-soluble ligands. Mar. Biol. 84: 41–45.

BIESINGER, K. E., AND G. M. CHRISTENSEN. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. 29: 1691–1700.

BORGMANN, U. 1983. Metal speciation and toxicity of free metal ions to aquatic biota, p. 47–72. *In* J. O. Nriagu [ed.] Aquatic toxicology. John Wiley & Sons, New York, NY.

BORGMANN, U., AND M. MUNAWAR. 1989. A new standardized sediment bioassay protocol using the amphipod *Hyalella azteca* (Saussure). Hydrobiologia 188/189: 425–531.

BORGMANN, U., K. M. RALPH, AND W. P. NORWOOD. 1989. Toxicity test procedures for *Hyalella azteca*, and chronic toxicity of cadmium and pentachlorophenol to *H. azteca*, *Gammarus fasciatus*, and *Daphnia magna*. Arch. Environ. Contam. Toxicol. 18: 756–764.

CONNOLLY, J. P. 1985. Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. 4: 573–582.

DAVIES, A. G. 1978. Pollution studies with marine plankton. Part II. Heavy metals. Adv. Mar. Biol. 15: 381–508.

FLORENCE, T. M., B. G. LUMSDEN, AND J. J. FARDY. 1983. Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom *Nitzschia closterium*. Anal. Chim. Acta 151: 281–295.

INTERNATIONAL JOINT COMMISSION. 1976. Cadmium, p. 22–32. Fourth Annual Report of the Great Lakes Water Quality Board, Appendix A. International Joint Commission, Windsor, Ont.

STEPHENSON, M., AND G. L. MACKIE. 1988. Multivariate analysis of correlations between environmental parameters and cadmium concentrations in *Hyalella azteca* (Crustacea: Amphipoda) from central Ontario Lakes. Can. J. Fish. Aquat. Sci. 45: 1705–1710.

1989a. A laboratory study of the effects of waterborne cadmium, calcium, and carbonate concentrations on cadmium concentrations in *Hyalella azteca* (Crustacea: Amphipoda). Aquat. Toxicol. 15: 53–62.

1989b. Net cadmium flux in *Hyalella azteca* (Crustacea: Amphipoda) populations from five central Ontario Lakes. Sci. Total Environ. 87/88. 463–475.

SUNDELIN, B. 1983. Effects of cadmium on *Pontoporeia affinis* (Crustacea:Amphipoda) in laboratory soft-bottom microcosms. Mar. Biol. 74: 203–212.

WINNER, R. W., AND J. D. GAUSS. 1986. Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. Aquat. Toxicol. 8: 149–161.

Metal Binding Activity of the Gills of Rainbow Trout (*Oncorhynchus mykiss*)

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Reid, S. D., and D. G. McDonald. 1991. Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1061–1068.

The objective of this study was to characterize the metal binding of the external gill surface in an attempt to interrelate metal chemistry, gill surface binding activity, and possibly metal toxicity. Individual gill arches from adult rainbow trout (*Oncorhynchus mykiss*) were exposed to various concentrations of either ^{140}La, ^{45}Ca, ^{109}Cd, or ^{64}Cu. The apparent dissociation constant (K_D) and capacity (B_{max}) of the external gill surface for the metals was calculated using Scatchard plot analysis. Half-saturation time ($t_{1/2}$) and binding cooperativity (H_n) were also determined. The K_D for gill metal binding ranged from 0.31 ± 0.02 mM for La^{3+} (high affinity) to 4.3 ± 0.04 mM for Cu^{2+} (low affinity). The gill binding affinity for Ca^{2+} was threefold lower than for La^{3+}, but was similar to that for Cd^{2+}. The gills had the lowest affinity for Cu^{2+}, almost 14 times lower than that for La^{3+}. B_{max} for La^{3+} was found to be significantly lower than for any other metal (0.14 ± 0.001 $\mu mol \cdot g^{-1}$), while nearly seven times as much Cu^{2+} was accumulated by the gills compared with La^{3+}. The Ca^{2+} and Cd^{2+} B_{max} estimates were intermediate.

On a tenté d'établir une relation entre, d'une part, la chimie des métaux, la liaison des métaux à la surface des ouïes et la toxicité des métaux et, d'autre part, la liaison des métaux à la surface externe des ouïes. On a donc exposé des branchicténies individuelles de truite arc-en-ciel (*Oncorhynchus mykiss*) adulte à diverses concentrations de ^{140}La, de ^{45}Ca, de ^{109}Cd et de ^{64}Cu. On a ensuite calculé la constante de dissociation apparente (K_D) et la capacité (B_{max}) de la surface externe des ouïes à l'aide de l'analyse du graphique de Scatchard, ainsi que le demi-temps de saturation ($t_{1/2}$) et le taux d'agglutination (H_n). La K_D des métaux liés aux ouïes allait de 0,31 ± 0,02 mM dans le cas de La^{3+} (affinité élevée) à 4,3 ± 0,04 mM dans le cas du Cu^{2+} (faible affinité). L'affinité du Ca^{2+} pour les ouïes était trois fois moins élevée que le La^{3+}, mais était semblable au Cd^{2+}. Les ouïes avaient moins d'affinité pour le Cu^{2+}, soit environ 14 fois de moins que pour le La^{3+}. La B_{max} du La^{3+} était nettement inférieure par rapport aux autres métaux (0,14 ± 0,001 $\mu mol \cdot g^{-1}$); de plus, les ouïes ont accumulé presque sept fois plus de Cu^{2+} que de La^{3+}. Les estimations de la B_{max} du Ca^{2+} et du Cd^{2+} étaient intermédiaires.

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Fish gills are physiologically complex and structurally delicate, and thus are an important target of waterborne toxicants. In a 250-g adult rainbow trout (*Oncorhynchus mykiss*), for example, the gills make up 60% of the total external surface area and are exposed, by ventilation, to over 48 L of water per hour (Reid 1989). The gill surface is covered with a layer of protecting mucus, consisting of glycoproteins, mucoplysaccharides, low molecular weight compounds, and water (Wold and Selset 1977; Fletcher et al. 1976; Van de Winkel et al. 1986), and like the bacterial cell wall (Beveridge and Murray 1980; Doyle et al. 1980), this mucus is likely to have an abundance of surface components which are negatively charged at environmental pH's. Therefore, the gills will attract waterborne metals. This is supported by the observations that, under conditions of acute exposure, the gills are the primary site of toxicity of several metals (Cu^{2+}, Lorz and McPherson 1976; Lauren and McDonald 1985; Cd^{2+}, Verbost et al. 1987, 1988; Reid and McDonald 1988; Zn^{2+}, Spry and Wood 1985; Al, Booth et al. 1988).

In an earlier study (Reid and McDonald 1988), we showed that Cd^{2+}, Cu^{2+}, and H^+ disrupt gill function in rainbow trout by impairing transepithelial ion exchange. More importantly, we observed qualitative differences amongst the effects of these metals. Since Cd^{2+}, Cu^{2+}, and H^+ undoubtedly react with biological ligands in fundamentally different ways, we hypothesize that the differences in toxicity might relate to one or more of their following properties: charge, ionic radius, ligand binding preference (e.g. oxygen versus nitrogen or sulphur centers), and binding affinity (Nieboer and Richardson 1980).

The objective of this study was to characterize the metal binding activity of the external gill surface and relate this to metal toxicity. A pharmacological approach to the binding of metals to the gills was used with the approach that the gill is a surface containing metal-binding ligands (metal "receptors"). The properties of the "receptors" were then characterized by the determination of key binding parameters: the apparent dissociation constant (K_D), binding capacity (B_{max}), time to half-saturation ($t_{1/2}$), and the mode (cooperativity, H_n) of binding. The metals chosen were lanthanum (La^{3+}), copper (Cu^{2+}), cadmium (Cd^{2+}), and calcium (Ca^{2+}). Whilst these metals exhibit a wide range of chemical properties (ionic radius, charge, molecular weight), all are known to disturb electrolyte balance in trout by the inhibition of active uptake and/or the stimulation of passive loss (La^{3+}, Freda and McDonald 1988; Perry and Flik 1988; Cu^{2+}, Lauren and McDonald 1985; Cd^{2+}, Verbost et al. 1987, 1988; Reid and McDonald 1988). The experimental protocol was established using La^{3+} as a model metal, since it was previously shown that La^{3+} binds to the external gill epithelial surface (McWilliams 1983) without penetrating to intracellular compartments (Weiss 1974; Perry and Flik 1988).

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Materials and Methods

Animals

Adult rainbow trout (weight range 235–287 g) were obtained from a commercial hatchery (Silver Springs Trout Farm, Petersburg, Ont.). The fish were held in a 400-L polyethylene tank continuously supplied with aerated, dechlorinated Hamilton tap water at 13–16°C (hard water: ~1 mmol $Ca^{2+} \cdot L^{-1}$, ~0.6 mmol $Na^{+} \cdot L^{-1}$). Trout were acclimated to the holding tank conditions for at least 2 wk prior to experimentation. Fish were fed daily to satiation with commercial trout pellets and the tank was siphoned regularly to prevent the build-up of organic debris.

Experimental Protocol

Fish were killed with a single blow to the head. Individual gill arches were carefully removed from the animals and placed immediately in a 0.9% NaCl solution at 15°C until all arches from one animal were removed and the metal radioisotope exposures could be initiated (2–3 min). Only the three most anterior gill arches from each side of the branchial basket were utilized from each fish in an attempt to maintain a standard gill weight (weight range 352–456 mg).

The exposure solutions (50 mL), containing radioisotopes of the appropriate metal, were held in 125-mL sample cups. The metals used in this study included La (as $LaCl_3$), Cd (as $CdCl_2$), Ca (as $CaNO_3$), and Cu (as $Cu(NO_3)_2$). The radioisotopes of Ca (^{45}Ca) and Cd(^{109}Cd) were purchased from New England Nuclear (NEN), while those of La (^{140}La) and Cu(^{64}Cu) were manufactured at the McMaster University Reactor. Each sample cup was fitted with an individual air line to provide constant mixing and was placed in a temperature-controlled water bath at 15 ± 1°C (Haake model D1 heater/recirculator). Assay solution pH was adjusted to 6.7 (range 6.53–6.80). Two 5-mL samples of assay solution were taken prior to gill exposure and two immediately after exposure for the determination of radioactivity and metal concentration.

Individual arches were removed from the saline and rinsed briefly in deionized water (15 s) to clean the external surface of the isolated gills of debris associated with removal from the animal. This was followed by a subsequent 15-s rinse in 5 mM ethylenediaminetetraacetic acid (EDTA), BDH Chemicals) following a procedure developed by McWilliams (1983) to remove Ca^{2+} and Mg^{2+} from the gills prior to the in vitro metal exposures. The EDTA was prepared as a potassium salt and neutralized to pH 7.7 with 0.1 N KOH. Preliminary experiments showed that 5 mM EDTA was optimal for obtaining maximal and reproducible metal binding. Excess EDTA was removed by quickly rinsing the tissue in another deionized water bath for 15 s.

Once cleaned, the arches were used in one of two separate metal binding activity experiments. One experiment characterized the time course of metal saturation of the external gill surface through the determination of $t_{1/2}$. The other experiment characterized the binding kinetics of the metals to the gill surface through the determination of K_D, B_{max}, and Hill coefficients (H_n) of the various metal – gill surface interactions. In both experiments, which were repeated at least twice, only one isolated gill was exposed to a metal radioisotope solution per sample cup, with a total of six sample cups in use at any one time.

Metal half-saturation times ($t_{1/2}$)

Isolated gills were exposed to 10^{-2} M radiometal solutions (10^{-3} M in the case of Cu^{2+}) for either 0, 15, 30, 60, 90, 150, 300, 600, 900, 1500, or 1800 s ($N = 4$ at each time). The specific activities of La^{3+}, Ca^{2+}, Cu^{2+}, and Cd^{2+} were 18.0, 54.0, 11.5, and 54.0 $kBq \cdot mol^{-1}$, respectively. Based on these estimates, a time of 300 s was chosen for experiments measuring the metal binding characteristics of the gills.

Metal binding characteristics (K_D, B_{max}, *and* H_n)

Additional isolated gills were exposed to one of nine radiometal concentrations ranging from 10^{-7} to 10^{-2} M (up to 10^{-3} M for Cu^{2+}) for exactly 300 s. The specific activities of La^{3+}, Ca^{2+}, Cu^{2+}, and Cd^{2+} were kept constant at 35.5, 106.7, 23.4, and 105.1 $kBq \cdot mol^{-1}$, respectively, over the range of exposure concentrations.

Following metal exposure, isolated gills were immediately rinsed for 15 s in deionized water to remove any loosely bound metal. For all metals, this brief rinse removed over 80% of the rapidly removable metal with only an additional 12% removed with a further 270 s of rinsing. Excess rinse water was then gently shaken off the gill, the gill filaments were removed from the cartilaginous gill arch, and the filaments were placed in preweighed 20-mL scintillation vials. The tissue was digested at room temperature for 7 d in 3 mL of 3 N nitric acid (Fisher Scientific). Gill tissue digests were vortexed several times daily to ensure complete tissue digestion.

Analytical Methods

Total metal bound to the gills was determined from the tissue digests using either liquid or solid scintillation techniques, depending on the metal radioisotope used. One-millilitre aliquots of tissue digests, prepared in triplicate, from gills exposed to either ^{140}La, ^{64}Cu, or ^{109}Cd were placed in 15 × 110 mm gamma counting tubes (John's Scientific) and counted on an Nuclear Chicago (model 1085) automatic gamma counting system with a 762 × 762 mm NaI(TI) crystal. Counts from samples containing ^{140}La (half-life = 40.9 h) or ^{64}Cu (half-life = 12.9 h) were corrected for decay.

Gill Ca (^{45}Ca) content was determined by mixing 100 μL of digest plus 4.9 mL of water with 10 mL of scintillation cocktail (ACS Amersham). Liquid scintillation samples, prepared in triplicate, were counted on a liquid scintillation counter (LKB Rackbeta) and automatically corrected for quench against standards of known radioactivity and variable concentrations of tissue digest.

In all cases, bound metal was calculated per gram of wet gill tissue according to the following equation:

$$(1) \quad \text{Bound metal } (\mu mol \cdot g^{-1}) = \frac{DPM \times DF \times Sa_w}{\text{tissue wet weight}}$$

where DPM is the radioactivity of the tissue digest corrected for background and quench, DF is a correction factor to account for the dilution of the tissue digest, and SA_w is the specific activity of the assay solution (kilobecquerels per micromole).

All assay solutions were prepared by serial dilution of concentrated (1 M) stock solutions. Metal concentrations were verified using atomic absorption flame photometry (Ca^{2+}, Cd^{2+}, Na^+, Cu^{2+}) and flame emission photometry (La^{3+}) on a Varian AA (model 1275) against known standards.

Data Analysis

Measurements of gill metal binding were analyzed using nonlinear regression (Duggelby 1981; Johnston 1985) and Scatchard plot techniques (Scatchard 1949). The $t_{1/2}$ was calculated from the time – metal accumulation data using nonlinear regression (Duggelby 1981). The metal binding curves (fixed exposure time, variable metal concentration) appeared to possess both saturable and nonsaturable components. Consequently, two methods of obtaining K_D and B_{max} estimates of the saturable component were employed. In the first method, estimates of K_D and B_{max} were obtained directly from the original (total) saturation curves using nonlinear regression which included a linear term according to the model of Johnston 1985):

$$(2)\quad \text{Total bound} = \frac{B_{max} \times [Me]}{K_D + [Me]} + (m \times [Me])$$

where m is the slope of the linear component which passes through the origin and [Me] represents the metal concentration. The second method consisted of a computed-based iterative curve-stripping technique where a linear component was removed according to the best fit of a Scatchard plot.

A Scatchard plot was generated by the following equation:

$$(3)\quad \frac{MeG}{Me} = -\frac{MeG}{K_D} + \frac{B_{max}}{K_D}$$

where Me is the free metal concentration (Free, micromoles per litre), Me*G* is the amount of metal bound (Bound) to the gill (*G*, micromoles per gram). The K_D (micromoles per litre) of the gill and the total B_{max} (micromoles per gram) of the gill for a specific metal are determined from the slope and *x*-intercept of the Scatchard plot, respectively, when Bound/Free is plotted against Bound. Good agreement was achieved between the methods. The latter method was used for all data analysis following the evaluation because it proved to be less time consuming.

Hill analysis (Hill 1910) was also employed to describe the nature of the metal binding according to the following equation:

$$(4)\quad \log[\%\ \text{total bound} \times (1 - \%\ \text{total bound})^{-1}] = H_n \times \log(Me)$$

where $[\%\ \text{total bound} \times (1 - \%\ \text{total bound})^{-1}]$ is the "receptor" fractional occupancy and H_n is the Hill coefficient obtained from the slope of the linear regression of log (Me) versus log (fractional occupancy). An H_n equal to 1 indicates independent binding of a ligand to a site or sites with one apparent affinity, while an H_n significantly greater than 1 indicates positive cooperativity among binding sites and an H_n significantly less than 1 indicates negative cooperativity or multiple binding sites.

Statistics

Data are expressed as means ± 1 SEM (*N*). Comparisons between specific binding characteristics ($t_{1/2}$, K_D, H_n) were made using a two-tailed, unpaired Student's *t*-test at a 95% level of confidence. Regression lines (Scatchard and Hill plots) were fitted by the method of least squares and the significance of the correlation coefficient (*r*) assessed. The coefficient of determination (R^2) was used as a measure of the amount of variation in the data fitted by both regression models. Lack of fit tests were performed on an HP-3000 (Hewlett Packard) using MINITAB (Minitab Inc.) to determine possible curvature in the data, possible lack of fit at outer *X*-values, and overall lack of fit of the model, with a level of confidence, in all cases, of 95%.

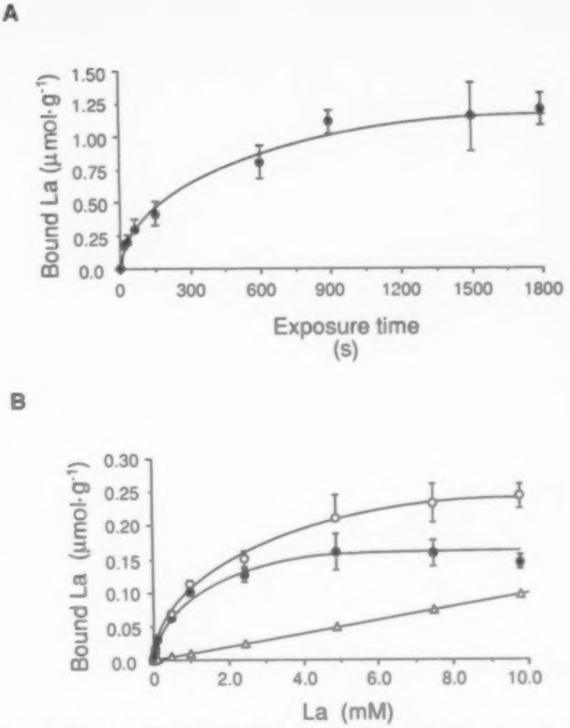

FIG. 1. Kinetics of gill La^{3+} binding. Data presented as means ± 1 SEM. (A) Time course for La^{3+} saturation of the gill microenvironment. Gills from hardwater-acclimated adults were removed, rinsed, and exposed to 10^{-2} M La^{3+} (see Materials and Methods for details). Each data point represents the average accumulation of La^{3+} by four independently exposed isolated gill arches ($N = 4$). The characteristic $t_{1/2}$ was determined from this curve using nonlinear regression analysis. (B) La^{3+} saturation curve. Data were generated by exposing isolated gills to a range of La^{3+} concentrations for exactly 300 s each (see Materials and Methods for details). Saturable (closed circles) and nonsaturable (open triangles) La^{3+} binding was calculated from total bound (open circles) using an iterative curve-stripping technique.

Results

Initial experiments were performed to determine the time course of metal saturation of isolated gills. saturation of the gill (at 10^{-2}M La^{3+}) occurred rapidly (Fig. 1). From triplicate determinations, the mean $t_{1/2}$ was 182±11.5 s. Similar $t_{1/2}$ times for the other metals were obtained (see below) and as a consequence, an exposure duration of 300 s was selected for the subsequent saturation kinetic experiments.

La^{3+} binding appeared to have a saturable and a nonsaturable component (Fig. 1B). The saturable La^{3+} binding data were

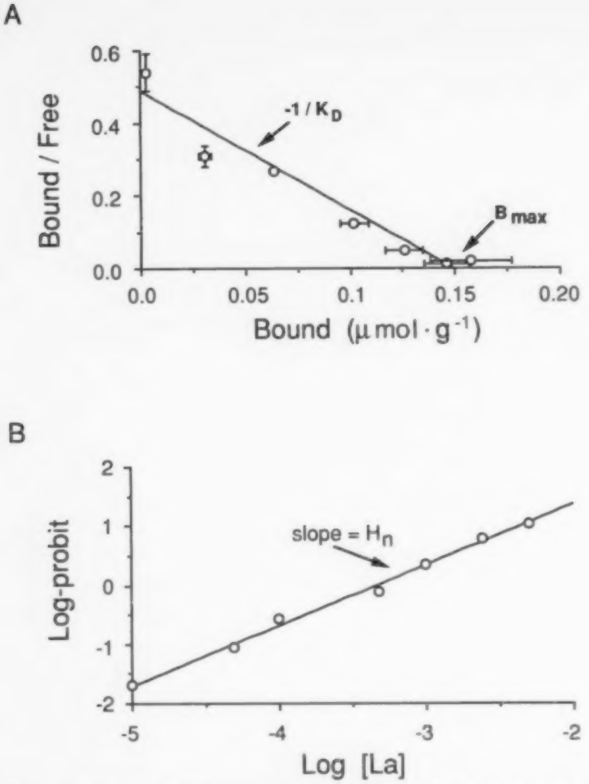

FIG. 2. Characteristic parameters of the binding of La^{3+} to isolated gills. The specific component of the La^{3+} saturation curve (Fig. 1B) was used to calculate the K_D, B_{max}, and H_n for gill La^{3+} binding. Data presented as means ± 1 SEM ($N = 4$). (A) A Scatchard plot for La^{3+}. The saturable component of the La^{3+} saturation curve (Fig. 1B) was transformed according to equation 2 for the determination of the K_D and B_{max} of gills for La^{3+}. The equation of the Scatchard plot was calculated using least squares linear regression ($Y = 0.50668 - 3.3232X$, $R^2 = 0.751$). The K_D and B_{max} are determined from the slope and x-intercept of this plot, as indicated on the figure. (B) A La^{3+} Hill plot. The specific component of a La^{3+} saturation curve of H_n. The equation of the Hill plot was calculated using least squares linear regression ($Y = 3.4052 + 1.02231X$, $R^2 = 0.994$). THe H_n represents the slope of the Hill plot, as indicated on the figure.

used to generate Scatchard plots from which both the K_D and B_{max} of the gills were determined (Fig. 2A). The x-intercept of this Scatchard plot yields an estimate of the maximum amount of metal that can be bound by the gill microenvironment (B_{max}), while the slope of the line is the negative inverse of the K_D. The La^{3+} saturation kinetic experiments were completed a total of three times (each concentration measured in triplicate), yielding total, specific, and nonspecific La^{3+} binding curves that were reproducible. Based on this analysis, the gill binding characteristics for La^{3+} were a K_D of 0.31 ± 0.02 mM and B_{max} of 0.14 ± 0.001 $\mu mol \cdot g^{-1}$ (Fig. 3B and 3C). The Hill plot (Fig. 2B) was not significantly different from unity at 1.04 ± 0.04 ($N = 3$, Fig. 3D), indicating no cooperativity amongst binding sites or alternatively that there was only one type of binding site on the gill.

The entire preceding protocol was then repeated for Cd^{2+}, Ca^{2+}, and Cu^{2+}. The $t_{1/2}$ estimates varied from 182 ± 11.5 s for La^{3+} binding to 148 ± 6.7 s for Cu^{2+} binding, with no estimate of $t_{1/2}$ significantly different from another (Fig. 3A). Similarly, no discernible differences between H_n for La^{3+}, Ca^{2+}, Cd^{2+}, and Cu^{2+} binding were found, although they ranged from 1.09 ± 0.04 for La^{3+} to 0.87 ± 0.09 for Cd^{2+} (Fig. 3D). However, marked differences between the K_D and B_{max} values for each metal were found.

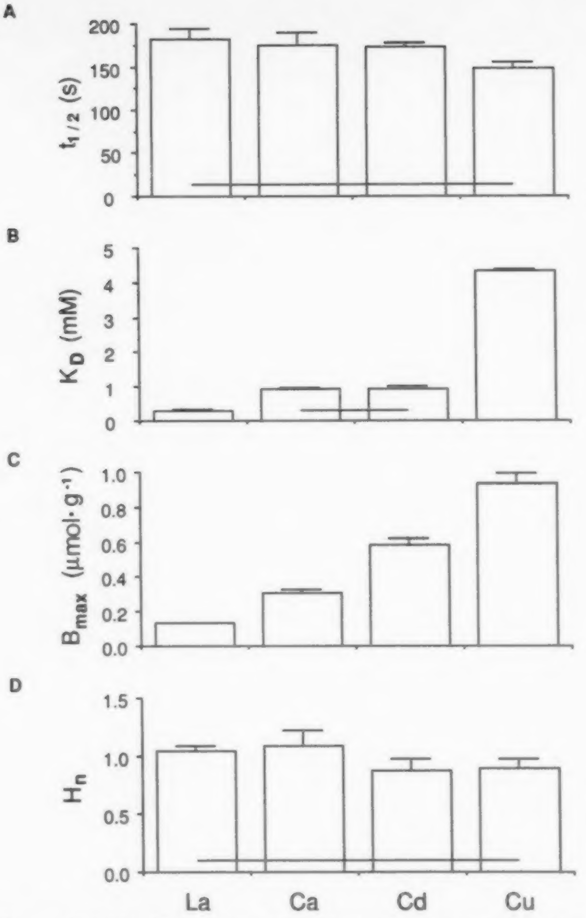

FIG. 3. Metal binding activity of the gill microenvironment. This histogram represents a comparison of the specific binding characteristics for La^{3+}, Ca^{2+}, Cd^{2+}, and Cu^{2+}. These data are presented as means of the three replicates for each binding characteristic ± 1 SEM ($N = 3$). The horizontal lines connect bars which are not significantly different from one another at a confidence limit of 95%.

The K_D for gill metal binding ranged from a low of 0.31 ± 0.02 mM for La^{3+} to a high of 4.3 ± 0.04 mM for Cu^{2+} (Fig. 3B). As affinity is inversely proportional to K_D, La^{3+} was bound by the gills with the highest affinity. The affinity of Ca^{2+} binding was found to be threefold lower than that for La^{3+}. However, the gills had nearly identical affinities for both Cd^{2+} and Ca^{2+}. The gills had the lowest affinity for Cu^{2+}, which was found to be 4.6 times lower than the affinity of Cd^{2+} or Ca^{2+} binding and almost 14 times lower than that for La^{3+}.

There appeared to be an inverse relationship between the affinity (K_D^{-1}) and B_{max}; the higher the affinity, the lower the B_{max} for that metal (Fig. 3C). The B_{max} of the gills for La^{3+} was found to be significantly lower than for any other metal, while nearly 7 times as much Cu^{2+} was accumulated by the gills compared with La^{3+}. The Ca^{2+} and Cd^{2+} B_{max} estimates were intermediate.

Discussion

Methodology

In defining the binding kinetics of the surface of the gills of trout, we have developed a method of titrating entire isolated gills with radioisotopes of various metals. McWilliams (1983) used a similar approach to investigate gill Ca^{2+} binding in trout, as did Freda and McDonald (1988) to study La^{3+} binding. However, our approach has made some improvements over previous studies. McWilliams (1983) loaded isolated gills with ^{45}Ca and measured the rate of loss of surficially bound Ca^{2+} using the dissociation rate (k_1) of Ca^{2+} as an estimate of the affinity of the gill for Ca^{2+}. In contrast, the present study determined the K_D for Ca^{2+} binding which was similarly used as an indicator of binding affinity. K_D and k_1 are related, as K_D is equal to the ratio of the dissociation (k_1) to association (k_2) rates (i.e. $K_D = k_1/k_2$; Titeler 1981). Both are direct measures of the nature of the metal–gill interactions, but the method utilized by McWilliams (1983) does not allow for the determination of H_n, $t_{1/2}$, or B_{max}. In addition, the protocol used by Freda and McDonald (1988), specifically, the in vivo exposure of whole fish to $^{140}La^{3+}$ as compared with the in vitro exposure of isolated gills to metals, does not take into account differences in ventilation frequency or volume between individual fish or the rapid reduction in free La^{3+} concentration due to very high nonspecific (nongill) binding to regions of the animal, such as the general body surface, the buccal cavity, and dislocated mucus. Therefore, our protocol minimizes the problems associated with the use of the whole animal and provides a more detailed analysis of metal – gill surface interactions than would be obtained using the protocol initially established by McWilliams (1983).

The resultant binding data were characterized according to models used in radioreceptor assays. However, in contrast with classic receptor–ligand binding, it is highly unlikely that the binding of metals to the gills involves specific "receptors," although one study has demonstrated the presence of a specific cation binding protein, calmodulin, in the mucus of one species of tilapia, *Sarotherodon mossambica* (Flik et al. 1983).

The gill surface was found to saturate rapidly when exposed to La^{3+} ($t_{1/2} = 182.1 \pm 11.5$ s, Fig. 3A), with the simple curvilinear nature of the binding curve suggesting titration of a single compartment. Exposure durations of about twice the $t_{1/2}$ (i.e. 300 s) were chosen for the saturation kinetics experiments. The rationale for this choice was that the isolation of the gill surface as a site of metal binding was possible only with relatively brief exposure times, particularly in the case of certain metals which bind to a greater variety of sites than just on the gill surface (i.e. metals other than La^{3+}). In addition, brief exposure times reduced probable morphological alteration of the isolated gills and any modifications of cation binding over the duration of the exposure. One consequence of the brief exposure times, however, is the inability to determine absolute binding characteristics. In radioreceptor assays, exposure times are usually set at longer than 1 h (12 times the $t_{1/2}$ of this study), since an equilibrium between the agonist and the receptor is generally reached within this time (Titeler 1981). Although this has not been done in the present study, the lack of a discernible difference in the $t_{1/2}$ estimates amongst the metals (Fig. 3A) means that at least relative differences in the binding characteristics of the metals can be assessed.

Microenvironment of the Gills

The complete chemical composition of the gill microenvironment has yet to be established, but many of the constituents of this region are known. The epithelial membrane, composed primarily of phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine; Bolis et al. 1984), is covered with a layer of mucus, a watery mixture of glycosaminioglycans (hyaluronic acid, chondroitin sulphate; Fletcher et al. 1976; Wold and Selset 1977; Van de Winkel et al. 1986). Of direct importance to the metal binding activity of the gills is the fact that the phosphate, carboxylate, and sulphate groups of the chemical constituents of the gill surface have pK's ranging from 1.4 to 4.0 (Seimiya and Ohki 1973; Dawson et al. 1986). In fact, based on competition between H^+ and Ca^{2+} for surface binding sites, as assessed by H^+ titration of $^{45}Ca^{2+}$-loaded isolated gills, the external gill surface has been shown to possess a pK of 3.6 (Reid 1989). Therefore, at environmentally relevant pH's (pH > 5), these groups will be fully ionized, resulting in a negatively charged gill surface and potential sites for metal–gill interactions (i.e. metal "receptors").

Gill–Metal Binding

This study clearly demonstrates that the binding activity of the gills for various metals can be characterized and that the gills exhibit relative differences in both the binding affinity and the binding capacity for various metals.

According to Nieboer and Richardson (1980), the relative binding affinity of a metal for biological ligands will be a function of the tendency of the metal to form ionic versus covalent bonds, and the particular chemistry of the ligand. The classification scheme of Nieboer and Richardson (1980) is best summarized and visualized by the two parameters which they call the ionic index and the covalent index. The ionic index, defined as

$$\text{Ionic index} = Z^2/r$$

where Z is the formal charge of the metal and r is the ionic radius, provides an indication of the tendency of metals to form ionic bonds with biological ligands. Metals with a strong tendency for ionic bond formation (class A metals: La^{3+}, Ca^{2+}) tend to bind to oxygen-containing ligands (i.e. carboxylate, carbonyl, alcohol, phosphate, or phosphodiester groups). The covalent index, on the other hand, is a measure of covalent bond formation, and is defined as

$$\text{Covalent index} = X_m{}^2 r$$

where X_m is the metal–ion electronegativity and r is the ionic radius. Metals with strong tendencies towards covalent interactions (class B metals) typically bind to nitrogen- or sulphur-rich ligands (i.e. sulphydryl, disulphide, thioether, heterocyclic nitrogen, or amino groups. In addition, there is a class of metals (borderline metals: Cd^{2+}, Cu^{2+}) that exhibit both class A and class B characteristics.

The rank order of binding according to decreasing affinity as measured in this study (Table 1) is as follows:

$$La^{3+} > Ca^{2+} = Cd^{2+} > Cu \text{ (measured)}.$$

The ranking of these metals by way of decreasing ionic indices would be

TABLE 1. Metal chemistry and toxicity summary.

Metal	K_d (mM)	B_{max} ($\mu mol \cdot g^{-1}$)	Formal charge	Ionic radius (nm)[a]	Atomic number[a]	96-h LC_{50} ($\mu mol \cdot L^{-1}$)	Ionic index (Z^2/r)[b]	Covalent index ($X_m{}^2r$)[b]
La	0.31	0.14	3+	0.106	57	—	8.5	1.3
Ca	0.93	0.31	2+	0.099	20	—	4.0	1.0
Cd	0.94	0.58	2+	0.097	48	0.3[c]	4.1	2.9
Cu	4.31	0.93	2+	0.076	29	0.3[d]	5.4	3.0

[a] Weast et al. (1984).
[b] Nieboer and Richardson (1980).
[c] Cusimano et al. (1986).
[d] Howarth and Sprague (1978).

$$La^{3+} >> Cu^{2+} > Cd^{2+} = Ca^{2+} > Cu^{+} \text{ (predicted).}$$

The ranking of these metals by decreasing covalent indices would be

$$Cu^{+} > Cu^{2+} > Cd^{2+} >> La^{3+} > Ca^{2+} \text{ (predicted).}$$

The close agreement between the ranking of the binding affinities of these metals and their respective ionic indices (except Cu^{2+}; see below) suggests that the metal binding to the gill surface is according to ionic, not covalent, interactions (Table 1). Furthermore, since ionic interactions are more characteristic of oxygen-rich ligands (Nieboer and Richardson 1980), these data also suggest that the dominant metal "receptors" of the gill microenvironment consist of oxygen-rich centers (carboxylate, sulphate, phosphate) as opposed to nitrogen or sulphur centers (amino nitrogen, sulphydryl groups).

In support of the hypothesis that metal–gill interactions are predominantly ionic in nature are the findings of Doyle et al. (1980) who, using similar methods of data analysis, also found differences in the affinity of the cell wall of *Bacillus subtilis* for various metals (Na^{+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Sr^{2+}, Zn^{2+}, Mg^{2+}). The bacterial cell wall and the gill surface can be considered analogous, since the bacterial cell wall is also through to be negatively charged and to accumulate metals due to an abundance of ionized carboxylate and phosphate groups (Beveridge and Murray 1980; Doyle et al. 1980). Using the binding affinities of Doyle et al. (1980) for these seven metals and ranking them in accordance with either their covalent or ionic indices, one finds that the affinity of binding is best correlated with the ionic tendencies of the metals (ionic: $r=0.85$ versus covalent: $r=0.34$, $N=7$ for both). Hille et al. (1975) investigated the influence of several divalent metals (and H^{+}) on the sodium activation of frog myelinated nerve fibres. One finds similarly that the effectiveness of the metals used by Hille et al. (1975) is best correlated with the ionic tendencies of the metals (ionic: $r=0.72$ versus covalent: $r=0.00$, $N=8$ for both). Nearly identical results are obtained using the data of Blaustein and Goldman (1968) (ionic: $r=0.74$ versus covalent: $r=0.00$, $N=8$ for both). The objective of the Blaustein and Goldman (1968) study was similar to that of Hille et al. (1975) with only the sources of the axon (lobster) and assortment of cations differing between studies.

The observation that Cu^{2+} binding is not predicted by its ionic index suggests either that Cu^{2+} reacts at the gill surface in a form other than Cu^{2+} or that the ionic index may not be entirely appropriate for predicting all metal–gill surface interactions. There are two reasons to rule out the former. First, Cu^{2+} is the predominant aqueous metal species at the pH of the in vitro exposures (pH 6.5). Cu^{2+} would make up approximately 70% of the total, with the remainder being $CuOH^{+}$ (Campbell and Stokes 1985; Cusimano et al. 1986). Second, Cu^{+} is 6 orders of magnitude less stable in aqueous environments than Cu^{2+} and has a tendency to autooxidize to Cu^{2+} (Buckingham 1973). Therefore, it is unlikely that Cu^{+} would be present at the gill surface.

Thus, the question is what other properties of Cu^{2+} might explain its weaker than expected binding affinity? The answer may well lie with the somewhat unique coordination chemistry of Cu^{2+}. Cu^{2+} typically has a coordination number of 4 which results in a square-planar coordination geometry, i.e. four donor atoms (preferably nitrogen and/or sulphur) are arranged around Cu^{2+} such that the bonds are located on the same plane in space. In contrast, all of the other metals of this study have coordination numbers ranging from 6 to 9 and consequently more complex coordination geometries (i.e. tetrahedral, octahedral. The weak binding of Cu^{2+} suggests that within the gill microenvironment, the preferred coordination geometry of Cu^{2+}, involving the available ligands, is not easily established. Consequently, one can conclude that the metal "receptors" at the gill surface are more likely multidentate ligands, with greater than four donor atoms (from carboxylate, sulphate, and phosphate groups) involved in the metal coordination. Since the gill microenvironment is thought to be a collection of flexible polyanions in an aqueous environment, the coordination of metals by multiple donor atoms is not an unreasonable interpretation.

The apparent relationship between B_{max} and affinity (Table 1) is surprising, as one would expect the number of binding sites to remain constant, even though binding affinities may vary, as the acclimation conditions of the animals were maintained, and therefore, the nature of the gill surface should not have been altered. The assumption that the total number of metal-binding sites is constant may very well be correct for La^{3+} and Ca^{2+} binding, but may not be appropriate for the binding of Cd^{2+} or Cu^{2+}. The differences in the B_{max} of the latter two might simply be an indication that these metals bind to different anionic groups or "receptors" and that the concentrations of these "receptors" are not the same as those that bind either La^{3+} or Ca^{2+}. In terms of ligand preference, both La^{3+} and Ca^{2+} specifically bind through oxygen. If one assumes that there is a fixed number of oxygen atoms available, then it would be expected that the binding capacity for La^{3+} would be approximately 33% lower than for Ca^{2+}. This calculation is based on the premise that one atom of La^{3+} would neutralize three anionic groups, compared with two by Ca^{2+}. This explains observed differences between La^{3+} and Ca^{2+} binding.

In contrast, since Cd^{2+} and Cu^{2+} are borderline metals, they share a tendency to bind to a more diverse selection of ligands than La^{3+} and Ca^{2+} (which bind exclusively to oxygen-rich ligands). Therefore, it would be expected that the gill binding capacity for Cd^{2+} and Cu^{2+} would be greater than for La^{3+} and Ca^{2+}, as the borderline metals bind to more sites on the gill, independent of binding affinity. Cu^{2+}, having the greater tendency to interact with nonoxygen donor atoms of the two borderline metals, would likely bind to more sites than Cd^{2+}, as was observed (Fig. 3C). A variety of binding sites may not necessarily be identified using Scatchard or Hill plots if the affinities of all sites are not significantly different and the additional binding sites are only a minor component of the total sites available for metal–surface interactions.

Mechanisms of Toxicity

The question posed now is in what way do the binding characteristics of the metals correlate with their toxicity? A low affinity of the gill surface for a metal can be expected to be more permissive of entry to the intracellular compartment. Once there, a variety of more complex binding sites are present, i.e. calmodulin, glutathione, metallothionein, and ATPases. Binding to these ligands could then result in any one or a combination of the following three types of toxic mechanisms: (1) blocking of the esssential biological functional groups of biomolecules, (2) displacing the essential metal ion in molecules, and (3) modifying the active conformation of biomolecules. These mechanisms of metal toxicity can be used to explain the specific inhibition of active ion transport resulting from both Cd^{2+} and Cu^{2+} exposure (Reid and McDonald 1988; Verbost et al. 1987, 1988; Lauren and McDonald 1987). Both are thought to bind to specific basolateral ATPases (Verbost et al. 1987, 1988; Lauren and McDonald 1987). Cu^{2+} is more disruptive to gill function than Cd^{2+} (Reid and McDonald 1988), likely because it has a stronger tendency to form covalent bonds with nitrogen/sulphur-rich centers such as those found within the ATPase itself (Lauren 1986). In addition, the affinity of the gill surface for Cu^{2+} is lower than for Cd^{2+} (Fig. 3C), which may indicate that the effective dose of Cu^{2+} in a previous study (Reid and McDonald 1988) was in fact greater than Cd^{2+} even though the concentrations were equimolar. This would result if, as shown by Part and Lock (1983), a greater surface binding affinity more effectively retards metal penetration than lower affinity binding. This interpretation is independent of the Cu^{2+} species present, since both Cu^{2+} and $CuOH^+$ are toxic (Lauren and McDonald 1985). Thus, Pagenkopf's (1983) contention that the greater the binding affinity, the greater the toxicity may only be correct for the interaction of a metal with the specific site at which the metal manifests its toxic effect, and not with the overall surface.

However, metals have also been shown to directly influence the ionic permeability of the branchial membrane, promoting substantial net ion loss which can lead to fish mortality (McDonald et al. 1988). This impact of metals on gill permeability has been attributed, in large part, to displacement of surface-bound Ca^{2+} (McWilliams 1983; Lauren and McDonald 1985; Booth et al. 1988; Freda and McDonald 1988; McDonald et al. 1988). The development of this particular toxic mechanism can be associated with the fact that Ca^{2+} stabilizes the epithelial membrane, consequently reducing ionic permeability (Hille et al. 1975; Steen and Stray-Pederson 1975; Oschman 1978). The displacement of Ca^{2+} and subsequent increase in permeability would occur only if a metal had an equal or greater relative affinity for the Ca^{2+} "receptors"; and in addition did not assume the membrane stabilizing function of Ca^{2+}. The phenomenon of direct competition between toxic trace metals and Ca^{2+} for gill binding sites is the focus of a subsequent study.

Conclusions

First, the application of radioreceptor assay techniques to characterize the interaction of the external gill surface and various metals has been successful in terms of identifying key properties of the surface and the metals. Second, differences in binding affinity and capacity of the gill can be correlated with some specific chemical properties of the individual metals, in particular their tendency to bind to the external surface through either ionic or covalent interactions. Finally, it is intriguing to speculate that the specific toxicity of a metal might be inversely related to tendency of the metal to bind to the external gill surface via ionic interactions, with the gill microenvironment acting as a buffer between the external and internal environments. Thus, the lower the affinity of the surface for a particular metal, the greater the likelihood of the metal disrupting intracellular processes which may lead to gill dysfunction.

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References

Beveridge, T. J., and R. G. E. Murray. 1980. Site of metal deposition in the cell wall of *Bacillus subtillus*. J. Bacteriol. 141: 876–887.

Blaustein, M. P., and D. E. Goldman. 1968. The action of certain polyvalent cations on the voltage-clamped lobster axon. J. Gen. Physiol. 51: 279–291.

Bolis, C. L., A. Cambria, and M. Fama. 1984. Effects of acid stress on fish gills, p. 122–129. *In* C. L. Bolis, A. Cambria, and M. Fama [ed.] Toxins, drugs, and pollutants in marine animals. Springer-Verlag, Berlin.

Booth, C. E., D. G. McDonald, B. P. Simons, and C. M. Wood. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

Buckingham, D. A. 1973. Structure and stereochemistry of coordination compounds, p. 3–63. *In* G. L. Eichhorn [ed.] Inorganic chemistry. Elsevier Science Publishing Co., New York, NY.

Campbell, P. G. C., and P. M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42: 2034–2049.

Cusimano, R. F., D. F. Brakke, and G. A. Chapman. 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to trout. Can. J. Fish. Aquat. Sci. 43: 1497–1503.

Dawson, R. M. C., D. C. Elliot, W. H. Elliot, and K. M. Jones. 1986. Data for biological research. Oxford University Press, New York, NY. 580 p.

Doyle, R. J., T. H. Mathews, and U. N. Streips. 1980. Chemical basis for selectivity of metal ions by the cell wall of *Bacillus subtillus*. J. Bacteriol. 143: 471–480.

Duggelby, R. C. 1981. A nonlinear regression program for small computers. Anal. Biochem. 110: 9–18.

Fletcher, T. C., R. Jones, and L. Reid. 1976. Identification of glycoproteins in goblet cells of epidermis and gill of plaice (*Pleuronectes platessa* L.), flounder (*Platichthys flesus* L.) and rainbow trout (*Salmo gairdneri* Richardson). Histochem. J. 8: 597–608.

Flik, G., J. H. van Rijs, and S. E. Wendelaar-Bonga. 1983. Evidence for the presence of calmodulin in fish mucus. Eur. J. Biochem. 135: 651–654.

Freda, J., and D. G. McDonald. 1988. Physiological correlates of interspecific variation in acid tolerance of fish. J. Exp. Biol. 136: 243–258.

Hill, A. V. 1910. The mode of action of nicotine and curari determined by the form of the contraction curve and the method of temperature coefficients. J. Physiol. 39: 361–373.

Hille, B. A., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near sodium channels of nerves: divalent ions, monovalent ions, and pH. Trans. R. Soc. Lond. Ser. B270: 301–318.

Howarth, R. S., and J. B. Sprague. 1978. Copper lethality to rainbow trout in waters of various hardness and pH. Water Res. 12: 455–462.

Johnston, A. 1985. SIMP: a computer program in BASIC for nonlinear curve fitting. J. Pharmacol. Methods 14: 323–329.

Lauren, D. J. 1986. Mechanisms of toxicity and acclimation to copper in trout. Ph.D. thesis, McMaster University, Hamilton, Ont.

Lauren, D. J., and D. G. McDonald. 1985. Effects of copper on branchial ion regulation in the rainbow trout, *Salmo gairdneri* Richardson. J. Comp. Physiol. 155B: 635–644.

1987. Acclimation to copper by rainbow trout, *Salmo gairdneri:* I. Physiology. Can. J. Fish. Aquat. Sci. 44: 99–104.

Lorz, H. W., and B. P. McPherson. 1976. Effects of copper or zinc in freshwater on adaptation to seawater and ATPase activity and the effects of copper on migratory disposition of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 33: 2023–2030.

McDonald, D. G., J. P. Reader, and T. K. R. Dalziel. 1988. The combined effects of pH and metals on fish ionoregulation, p. 221–242. *In* R. Morris, E. W. Taylor, and J. A. Brown [ed.] Acid toxicity and aquatic animals. SEB Seminar Series. Vol. 31. Cambridge University Press, London.

McWilliams, P. G. 1983. An investigation of the loss of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. Comp. Biochem. Physiol. 74A: 107–116.

Nieboer, E., and D. H. S. Richardson. 1980. The replacement of the nondescript term ‘heavy metals’ by a biologically and chemically significant classification of metal ions. Environ. Pollut. 1B: 3–26.

Oschman, J. L. 1978. Morphological correlates of transport, p. 55–84. *In* G. Geisbisch, D. C. Tosteson, and H. H. Ussing [ed.] Membrane transport in biology. Vol. III. Transport across multi-membrane systems. Springer-Verlag, New York, NY.

Pagenkopf, G. K. 1983. Gill surface interaction model for trace metal toxicity to fish: role of complexation, pH, and water hardness. Environ. Sci. Toxicol. 17: 347–352.

Part, P., and R. A. C. Lock. 1983. Diffusion of calcium, cadmium and mercury in a mucous solution from rainbow trout. Comp. Biochem. Physiol. 76C: 259–263.

Perry, S. F., and G. Flik. 1988. Characterization of branchial transepithelial calcium fluxes in freshwater trout, *Salmo gairdneri*. Am. J. Physiol. 254: R491–298.

Reid, S. D. 1989. Metal-gill surface interactions in rainbow trout, *Oncorhynchus mykiss*. Ph.D. thesis, McMaster University, Hamilton, Ont.

Reid, S. D., and D. G. McDonald. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 244–253.

Scatchard, G. 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 660–672.

Seimiya, T., and S. Ohki. 1973. Ionic structure of phospholipid membranes, and binding of calcium ions. Biochim. Biophys. Acta 298: 546–561.

Spry, D. J., and C. M. Wood. 1985. Ion flux, acid-base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

Steen, J. B., and S. Stray-Pederson. 1975. Permeability of fish gills with comments on the osmotic behavior of cellular membranes. Acta Physiol. Scand. 95: 6–20.

Titeler, M. 1981. Understanding receptor binding assays, p. 39–73 *In* E. Marks and R. Rodnight [ed.] Methods in neurochemistry. Vol. 5. Pleneum Press, New York, NY.

Van de Winkel, J. G., T. H. M. S. M. Van Kuppevelt, H. M. J. Janssen, and R. A. C. Lock. 1986. Glycosaminoglycans in the skin mucus of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 85B: 473–475.

Verbost, P. M., G. Flik, R. A. C. Lock, and S. E. Wendelaar-Bonga. 1987. Cadmium inhibition of Ca^{++} uptake in rainbow trout gills. Am. J. Physiol. 253: R216–221.

1988. Cadmium inhibits membrane calcium transport. J. Membr. Biol. 102: 97–104.

Weast, R. C., M. J. Astle, and W. H. Beyer [ed.] 1984. C.R.C. handbook of chemistry and physics. 65th ed. C.R.C. Press, Inc., Boca Raton, FL. p. F-165.

Weiss, G. B. 1974. Cellular pharmacology of lanthanum. Annu. Rev. Pharmacol. 14: 344–356.

Wold, J. K., and R. Selset. 1977. Glycoproteins in the skin mucus of the char (*Salmo alpinus* L.). Comp. Biochem. Physiol. 56B: 215–218.

Habitat Selection by Stream-Dwelling Predatory Stoneflies

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Patterns of substrate size preference of predatory stoneflies were measured in a western Colorado, USA, stream and associations were examined between substrates and other physical and biological variables. Predatory *Megarcys signata* (Perlodidae) were found disproportionately on large stones that were not displaced during spring runoff. Predator densities were not consistently correlated with any other physical or biological variables measured. Prey densities were neither correlated with other physical variables nor with their own resource levels, with the exception that shredders were positively correlated with their relatively rare detrital resource. Interestingly, five prey taxa were autocorrelated, indicating that they recolonized, at similar densities, the same stones replaced in the same spots in the stream. Association analysis among the five most abundant taxa of mayfly grazers revealed that stones favorable for one grazer taxon were often favorable for others, but different grazer species rarely occupied distinctly different stones. Patterns observed suggest that predators are persistent enough to avoid negative associations with prey, yet prey taxa are mobile enough to avoid positive associations with predators. Further, habitat selection by predatory stoneflies may have evolved as a response to pressure from their own predators rather than as an adaptation for efficient prey acquisition.

On a étudié la taille du substrat préféré des plécoptères prédateurs dans un cours d'eau de l'ouest du Colorado (É.-U.) ainsi que les associations entre les substrats et d'autres variables physiques et biologiques. On a trouvé le perlodidé prédateur *Meragcys signata* dans une mesure disproportionnée sur les grosses pierres qui n'avaient pas été déplacés durant la fonte printanière. On n'a constaté aucune corrélation constante entre la densité de prédateurs et d'autres variables physiques ou biologiques. La densité de proies n'était pas non plus en corrélation avec d'autres variables physiques, ni avec l'abondance des ressources pour les proies, exception faite des déchiqueteurs, pour lesquels on a constaté une corrélation positive avec l'abondance de détritus, ressources relativement rare. Fait intéressant à noter, chez les proies, cinq taxons étaient en autocorrélation, ce qui signifie qu'il y avait recolonisation à densité comparable des pierres replacées au même emplacement dans le cours d'eau. L'analyse des associations entre les cinq taxons d'éphéméroptères brouteurs les plus abondants a révélé que les pierres favorables pour un taxon l'étaient souvent pour d'autres également; il était rare que des brouteurs d'espèces différentes occupent des pierres vraiment différentes. D'après les observations, les prédateurs seraient assez persistants pour qu'il n'y ait pas d'associations négatives avec les proies, tandis que les taxons de proies seraient assez mobiles pour qu'il n'y ait pas d'associations positives avec les prédateurs. En outre, le choix de l'habitat chez les plécoptères prédateurs pourrait être le résultat de la pression exercée par leurs propres prédateurs plutôt que d'une adaptation visant à faciliter l'obtention de proies.

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Despite the reportedly rare occurrence of randomness in the spatial distribution of plants and animals (Taylor et al. 1978), recent studies have demonstrated random dispersion patterns of benthic invertebrate predators foraging on patchily distributed prey (Walde and Davies 1984; Peckarsky 1988; Rasmussen and Downing 1988). Further, those and other studies have shown a lack of correlation between densities of benthic predators and prey (Feltmate et al. 1986), especially when prey species are highly mobile (Malmqvist and Sjöström 1984). This reported absence of an aggregative response of predators to patches of high prey density has been attributed to interference competition between predators (Walde and Davies 1984; Peckarsky and Penton 1985; Peckarsky 1991) and to the ephemeral nature of patches of highly mobile prey (Peckarsky 1988).

The lack of response by stream-dwelling predatory stoneflies (Perlodidae and Perlidae) to natural (Walde and Davies 1984; Peckarsky 1988) and experimentally manipulated (Peckarsky and Dodson 1980) prey patches suggests that prey organisms (largely Ephemeroptera, Simuliidae, Chironomidae) are sufficiently mobile to avoid positive associations with predators, and yet stoneflies are sufficiently persistent to prevent negative associations with prey (Rasmussen and Downing 1988). Thus, observed stonefly distributions are the consequence of a complex "behavioral arms race" (Sih 1984; Formanowicz and Bobka 1989) between predators and prey, and among competing predators. The questions remain, however, (1) whether stoneflies exhibit any consistent patterns that indicate the use of physical habitat cues by which they select habitat for foraging and (2) whether use of those cues improves their probability of locating prey-rich patches of substrate.

Many studies have been published on substrate size or heterogeneity preferences of predatory stoneflies (see Minshall 1984 for a review), with conflicting results ranging from no measurement of preference (Culp et al. 1983; Walde 1985) to preference for relatively small particle sizes (Reice 1980), medium-sized substrates (Feltmate and Pointing 1986; Feltmate et al. 1986), large substrates (Reice 1980), heterogeneous substrates (Williams 1980), or dark-colored substrates (Feltmate and Williams 1989). The purpose of this study was, first, to determine whether predatory stoneflies exhibited any patterns of preference for particular sizes of available substrates. The second goal was to develop hypotheses on the biological significance of substrate selection by determining whether other variables (physical and biological) were consistently associated with substrates favored by stoneflies (e.g. high probability of finding prey, suitable flow regime, and refuge from predation).

Methods

The study was carried out at a third-order riffle of the East River, Gunnison County, Colorado, a high-elevation (2940 m) snowmelt trout stream (38°57′30″N, 106°59′15″W) that flows through the site of the Rocky Mountain Biological Laboratory. The substrate at this site is heterogeneous cobble underlain by gravel and finer sediments. Thirty quadrat samples taken in July 1988 showed that surface sediments were composed of about 70% cobble, 20% pebbles, and 10% gravel. The geological composition of stones is highly diverse, with about 50% metamorphic slate, 33% igneous granite, and the remainder a mixture of other metamorphic stones (mostly marble), sedimentary sandstone, and sedimentary conglomerate.

Benthic Sampling

On three dates (at 2- to 3-wk intervals) during summers 1985, 1986, and 1987, 30 (largest feasible sample size) stones were selected randomly from the stream bottom and sampled for predatory stoneflies and other associated variables (Tables 1 and 2). All stones sampled were in the cobble size range (diameters 5–20 cm), since previous observations (unpublished) suggested that predatory stoneflies were rare on smaller substrates in this stream. Stone surface area was estimated in 1985 by wrapping stones in commercial aluminum foil with a known mass per unit area and then weighing the foil (Reice 1980). This method was changed thereafter, since predatory stoneflies show a preference for undersides of stones (Feltmate and Pointing 1986; B. L. Peckarsky, unpubl. data), and most prey taxa are found either primarily on tops or primarily on the bottoms of stones (B. L. Peckarsky, unpubl. data). During 1986 and 1987 the "birds-eye" top surface area (Fig. 1) was approximated by tracing stones on acetate sheets (McAuliffe 1984) as a more appropriate unit area from which to calculate predator and prey densities. For purposes of comparison with 1986–87 data, the 1985 total stone surface area measurements were divided by 4 to convert them to estimated top surface areas, assuming that stones were spheres (total surface area = $4\pi r^2$) and that the horizontal surface area was that of a circle ($=\pi r^2$). Since these stones were shaped more like ellipses (flattened) than spheres, 1985 estimated top surface areas are probably too small (Fig. 1). However, all analyses were done on each date separately, and no between-year comparisons were made. Therefore, underestimates of 1985 top surface areas (leading to overestimates of benthic densities, Table 2) do not affect the statistical outcomes or conclusions of this study.

In 1985 and 1986, prior to removal of stones from the stream, water depth to the surface of each stone was recorded, and the surface flow immediately above each stone was measured with a Marsh-McBirney model 201 flowmeter. Interstitial flow rates were estimated after invertebrate samples were taken by replacing each stone at its precise original location with a dried, preweighed plaster of Paris hemisphere (1 cm in diameter) attached by rubber bands to its undersurface. After 5–7 h, the hemispheres were retrieved and dried to a constant weight and inter-

TABLE 1. Summary of physical factors and resource levels (mean (± SE) or range (minimum–maximum); — no data).

Date	Stone surface area (cm^2) Minimum	Stone surface area (cm^2) Maximum	Surface	Interstitial flow (cm/s)	Water depth (cm)	Detritus (gdw/m^2)	Periphyton (10^3 cells/cm^2)	μg Chl *a*/cm^2
1985								
20 June	46[a]	656[a]	101.6	35.4	14.1	17.1	—	—
(n = 29)			(5.8)	(1.6)	(0.8)	(3.8)		
16 July	63[a]	446[a]	82.8	47.6	8.3	8.9	170.3	—
(n = 30)			(3.6)	(1.8)	(0.7)	(2.1)	(40.1)	
30 July	63[a]	446[a]	46.0	50.9	4.7	11.6	203.1	—
(n = 30)			(4.1)	(5.1)	(0.8)	(3.2)	(23.6)	
1986								
3 July	101	610	103.0	29.8	14.3	39.0	—	—
(n = 30)			(4.7)	(2.3)	(0.8)	(3.4)		
24 July	101	610	81.9	39.8	7.3	47.5	—	—
(n = 30)			(5.1)	(2.9)	(0.9)	(5.5)		
14 August	101	610	51.1	41.3	4.2	61.3	—	—
(n = 30)			(3.7)	(2.3)	(0.5)	(8.8)		
1987								
12 August	61	616	—	—	—	—	—	0.216
(n = 29)								(0.039)
26 August	76	616	—	—	—	—	—	0.169
(n = 30)								(0.017)
9 September	74	504	—	—	—	—	—	0.271
(n = 28)								(0.039)

[a] Approximated from total surface area ($4\pi r^2 - 4 = \pi r^2$ = approximate top surface area).

TABLE 2. Summary of mean benthic densities (± 1 SE) per square metre of top stone surface area (1985 values may be overestimates, since they are based on underestimated top surface area; — = no data).

Date	*Megarcys signata*	*Baetis bicaudatus*	*Cinygmula mimus*	*Epeorus deceptivus*	*Ephemerella infrequens*	*Rhithrogena* spp.	Simuliidae	Chironomidae	*Zapada haysi*	Total benthos
1985										
20 June	15.2	872.0	296.4	65.2	94.0	146.4	76.0	421.2	86.8	2215.6
(n = 29)	(5.6)	(119.2)	(61.6)	(14.0)	(22.8)	(48.0)	(21.6)	(100.4)	(21.2)	(275.6)
16 July	41.2	590.4	270.0	434.8	125.2	258.0[b]	277.6	102.0	168.8	2532.8
(n = 30)	(9.6)	(72.8)	(36.8)	(72.8)	(19.6)	(49.6)	(141.6)	(33.6)	(37.2)	(214.4)
30 July	26.8	1243.2[a]	191.2	798.4	104.4	142.0[b]	682.0	302.8	851.2	4504.0
(n = 30)	(8.4)	(161.6)	(23.6)	(76.4)	(14.4)	(22.8)	(296.0)	(62.8)	(152.0)	(616.0
1986										
3 July	53.0	352.5	240.8	269.5	132.4	269.8[b]	27.2	271.3	99.5	1949.0
(n = 30)	(12.6)	(62.1)	(40.9)	(96.5)	(18.3)	(51.6)	(9.9)	(43.7)	(28.7)	(219.0)
24 July	126.0[c]	733.9	196.4	451.8	567.4	259.4	372.0	1384.0	404.2	4813.0
(n = 30)	(24.5)	(91.8)	(25.1)	(53.3)	(86.6)	(44.2)	(147.0)	(161.0)	(83.0)	(483.0)
14 August	216.4[c]	941.7[a]	113.5	909.3	486.6	343.7[d]	160.4	1105.0	793.0	5174.0
(n = 30)	(43.5)	(92.3)	(17.7)	(76.5)	(54.0)	(76.9)	(41.0)	(102.0)	(105.0)	(312.0)
1987										
12 August	2164.0[c]	638.0[a]	21.0	427.2	719.0	304.3[d]	720.0	3595.0	—	—
(n = 29)	(464.0)	(106.0)	(5.9)	(44.5)	(110.0)	(50.9)	(167.0)	(567.0)		
26 August	2753.0[c]	2022.0	18.8	197.5	621.5	455.8[d]	3068.0	2659.0	—	—
(n = 30)	(305.0)	(168.0)	(7.3)	(24.9)	(86.1)	(58.9)	(571.0)	(397.0)		
9 September	1631.0[c]	1839.0	0	108.8	752.0	519.4[d]	1641.0	2342.0	—	—
(n = 28)	(206.0)	(225.0)		(21.8)	(101.0)	(53.2)	(592.0)	(515.0)		

[a] Summer cohort.
[b] *R. hageni.*
[c] Small *Megarcys.*
[d] *R. robusta.*

stitial flow was estimated from a regression equation ($y = 2.07x$) established previously between weight loss (x = grams per hour) and current velocity (y = cm/s) (Peckarsky and Penton 1990).

Invertebrate samples were obtained by placing an aquatic D-frame net (meshes = 250 μm) downstream of each stone while lifting it out of the stream, so that more loosely associated insects were washed into the net. Stones were then placed in pans in the same configuration as in the stream while horizontal surface areas were traced, and remaining insects were removed by hand. Insects were preserved in 70% EtOH, sorted, and identified to species under a dissecting microscope in the laboratory. Head capsule width of each individual was measured for 1985 and 1986 samples using an ocular micrometer.

Detritus associated with each stone was also collected with the invertebrates in 1985 and 1986, sorted from samples in the field and sieved through 1-mm mesh (eliminating fine particles), dried, and weighed for determination of resource levels available to benthic invertebrate shredders. Finally, after removal of all detritus and insects, stones were immersed in water and periphyton was sampled from a 16-cm^2 area of the upper surface using a device described by Hamala et al. (1981) and illustrated by Mayer and Likens (1987). The sampling device consisted of a portable two-speed drill attached to a peristaltic pumphead fitted with plastic tubing. Compressible weather stripping on the end of a Plexiglas cylinder sealed the area to be sampled, preventing contamination from adjacent areas and/or loss of loosely attached algal forms. The plastic tubing was inserted into the cylinder close to the substrate, and using the power of the drill to turn the peristaltic pump, the substrate was "vacuumed" and the water containing the periphyton was simultaneously pumped into a 500-mL sampling container. In 1985, periphyton samples were subsampled in the laboratory for taxonomic identification and algal cell counts using a Palmer cell and phase-contrast microscope. During 1987, periphyton samples were stored in an ice chest, returned to the laboratory, and filtered immediately through Whatman GF/F glass fiber filters in a 47-mm Millipore filtration unit. Filters were hand-ground and extracted in 90% EtOH for 6–24 h and refiltered for clarification. Fluorescence was read in a 75 × 12 mm cuvette on a Turner fluorometer, and concentrations of chlorophyll *a* and phaeophytin *a* were calculated. This procedure is presented in greater detail in Cowan and Peckarsky (1990).

Substrate Disturbance Experiment

In order to determine the relationship between stone size and the probability of being dislodged by snowmelt runoff, on 17 September 1987, after the stream bed had dried on a substantial portion of the riffle sampled, the top surface area of 90 stones (9 each along 10 transects) was traced on an acetate sheet, as in the above procedure. However, stones were not removed from the stream, but measured in their "natural lie" in the stream bed. Thus, the probability of later disturbance by current was not altered by handling and replacement of stones. Stones were marked (number coded) with oil-based paint, their locations mapped along the permanent transects, and the type of stone and whether or not stones were embedded in finer sediments were recorded. Top surface areas of stones sampled ranged from 28 to 1156 cm^2 (smaller and larger than those sampled in the previous procedure, Fig. 1; Table 1) with a mean (± SE) top surface area of 250 ± 25 cm^2. The next summer (22 July 1988), stones were retrieved by checking their original positions and noting the presence or absence of marked stones. Drawings of the missing stones were compared with stones then

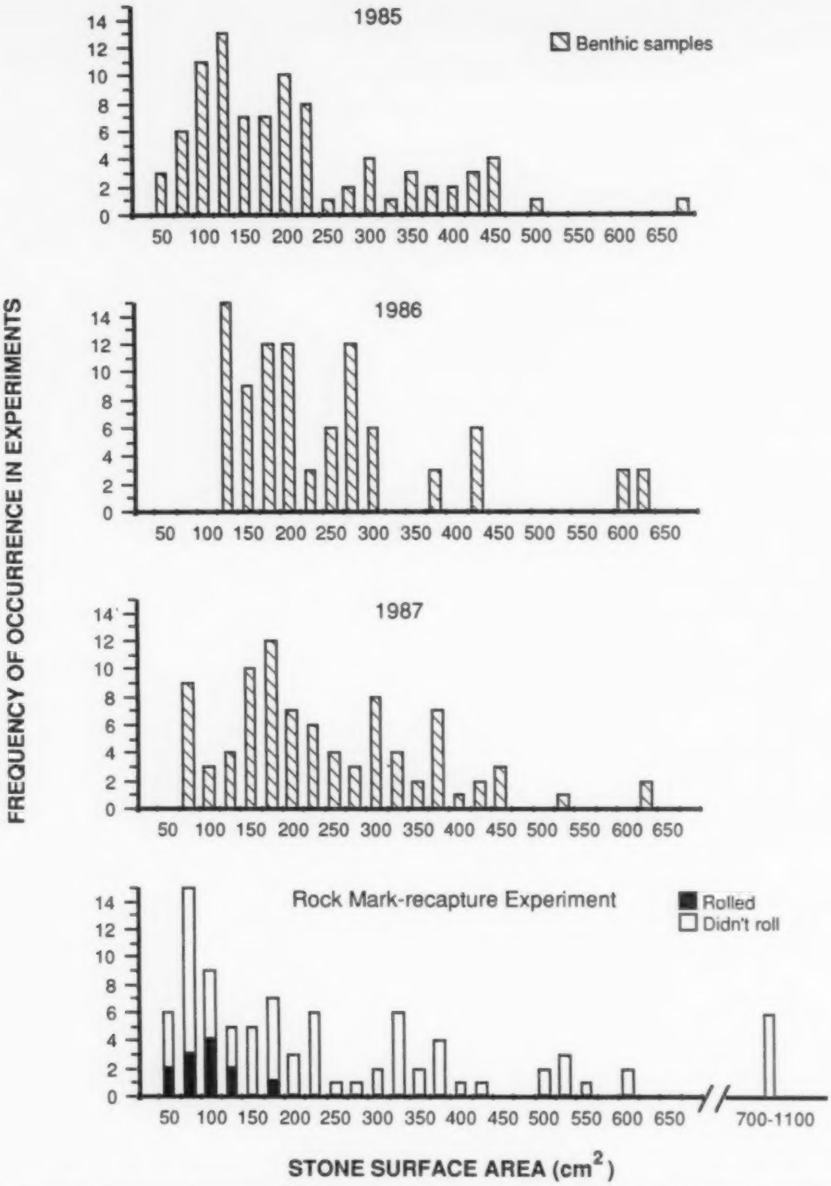

FIG. 1. Size frequency distributions (top surface area) of stones sampled from the field (benthic samples) during 1985–87 and of stones marked in September 1987 and retrieved in July 1988. The 1985 top surface areas were estimated from total surface areas.

present at those locations to ensure that absence was not erroneously concluded from numbers having worn off over the winter. Although many numbers were faint, in all cases at least a trace of paint was still present.

Questions and Statistical Analyses

Nonparametric statistics were used, since the data were most appropriate for analysis by distribution-free methods. Tests were conducted to answer four questions, which provided information on the potential biological significance of substrate preferences of stoneflies.

(1) With what physical factors were the densities of predatory stoneflies associated? Observed numbers of late-instar (present in June and July) and early-instar (present in August and September) *Megarcys signata* (Hagen) (Perlodidae) on stones greater than and less than 200 cm^2 top surface area (= approximately 16 cm in diameter) were compared by chi-square analysis with those expected given no preference for stone size. Expected frequencies were calculated on the basis of proportion of total surface area represented by each surface area category. Densities of stoneflies on slate versus granite substrates were compared using a Mann-Whitney *U* test. Associations between density of *Megarcys* (per square metre of top surface area) and

surface flow (centimetres per second), interstitial flow (centimetres per second), and water depth (centimetres) (Table 1) were determined using nonparametric correlation analysis (Spearman's R_s).

(2) Were densities of predatory stoneflies associated with biological factors such as densities of prey populations or abundance of food resources of prey populations? Spearman rank correlation coefficients were calculated between densities of *Megarcys* and densities and mean sizes of eight major prey taxa (Table 2) and between *Megarcys* and cell counts (per square centimetre) of periphyton (1985), micrograms of chlorophyll *a* per square centimetre (1987), and with grams dry weight of coarse detritus per square metre, (1985, 1986) (Table 1).

(3) Given that a relationship was observed between predatory stonefly densities and stone size, what other factors varied predictably with stone size?, i.e. by selecting stones of a certain size, what were the associated habitat features? Stability of stones greater than and less than 200 m^2 top surface area was assessed by comparing (chi-square analysis) the observed with expected frequencies of stone overturn given random probability of disturbance over stone size categories. Spearman rank correlation coefficients were calculated for stone size (top surface area) versus other physical factors (flow and depth) and stone size versus biological factors (prey density and prey resource abundance (periphyton and coarse detritus).

(4) Finally, were densities of prey populations associated predictably with any physical variables (stone size, flow, water depth) or biological variables (food resource levels: detritus or periphyton)? Spearman's R was used to determine the degree of association between densities of the eight major prey taxa and those variables. Because the same stones were resampled from the second to third sampling periods in 1985 and on all three dates in 1986, correlation analysis was also used to determine whether densities of each prey taxon were repeatedly high or low on the same stones sampled at 3-wk intervals. In other words, significant autocorrelations would indicate that after a disturbance the same species recolonized the same stones at similar densities. Interspecific correlations were also examined between pairs of the five major mayfly taxa, which are primarily grazing periphyton in this stream (Table 2). This analysis provided information on whether different species of the same functional group were attracted to the same stones.

Results

(1) Predatory Stoneflies and Physical Factors

Early-instar *Megarcys* were either distributed randomly with respect to stone surface area (1985) or overrepresented on stones with top surface area <200 cm^2 (1986, 1987), while late instars were significantly overrepresented on stones with top surface area >200 cm^2 (Table 3). Predator densities were not consistently correlated with surface flow, interstitial flow, water depth, or stone type (slate or granite). On only one date each was *Megarcys* density positively correlated with surface flow ($R_s = 0.386, p < 0.05$. $n = 30$, 30 July 1985) and interstitial flow ($R_s = 0.525$, $p < 0.05$, $n = 30$, 16 July 1985) or negatively correlated with water depth ($R_s = -0.502$, $p < 0.05$, $n = 30$, 16 July 1985). Scatter plots of *Megarcys* density versus physical variables indicated no potential for more complex relationships (e.g. preference for medium values). Thus, the only physical factor that was consistently associated with *Megarcys* density was stone size.

(2) Predatory Stoneflies and Biological Factors

Densities of *Megarcys* were significantly positively associated with those of prey taxa only when early-instar predators predominated in samples (see Peckarsky 1988 for 1985 and 1986 correlation coefficients). In 1987 (12 August, 26 August, and 9 September), early-instar *Megarcys* densities were significantly positively correlated with those of early-instar *Baetis bicaudatus* Dodds ($R_s = 0.809$, 0.795, and 0.765 on all three dates, respectively), *Epeorus deceptivus* (McDunnough) ($R_s = 0.518$ and 0.607 on the last two dates, respectively), *Ephemerella infrequens* McDunnough ($R_s = 0.594$ and 0.479 on the first and last date, respectively), Chironomidae ($R_s = 0.777$, 0.676, and 0.726 on all three dates, respectively), and Simuliidae ($R_s = 0.565$ and 0.388 on the first and last date, respectively). (Note that $R_{s0.05} = 0.355$ and $R_{s0.01} = 0.456$ for $n = 30$.) Despite this apparent predator aggregative response, early-instar *Megarcys* do not feed primarily on insect prey, having mostly periphyton in their stomachs (Allan 1982; B. L. Peckarsky, unpubl. data). Thus, this association between small *Megarcys* and the mayflies and dipterans was not due to an active ecological interaction, but rather to common association with the same stones (habitat heterogeneity sensu Hart 1979).

A positive association between predator densities and the resource levels of their prey (periphyton or detritus) would indicate that predators may choose habitat based on abundance of a sessile resource of their mobile prey. In this study there was no such relationship between predator densities and the resources of their prey on any sampling date. Thus, predators in this stream did not aggregate on their prey's resource.

(3) Factors Associated with Stone Size

Stones with a top surface area >200 cm^2 did not move over the winter and subsequent spring snowmelt period of 1988 (Fig. 1). Stones <200 cm^2 in surface area moved with 25% probability (Table 4). Also, the mean size of stones that moved (100 ± 13 cm^2) was significantly smaller than that of stones that did not move (275 ± 28 cm^2) Mann-Whitney U test, $p < 0.05$). Whether the stones were embedded in fine substrates did not alter their probability of having moved over the winter. Thus, the stone size category preferred by late-instar predatory *Megarcys* was significantly more stable with respect to probability of overturn during periods of high flow. Larger stones were also shallower on three of six dates (20 June 1985, 30 July 1985, and 3 July 1986, $R_s = -0.483$, -0.401, and -0.486, respectively) and were associated with faster interstitial flows on two of six dates (20 June 1985 and 30 July 1985, $R_s = 0.403$ and 0.488, respectively).

Contrary to previous reports (McAuliffe 1984), larger substrates did not have more periphyton, measured as cells per square centimetre or micrograms of chlorophyll *a* per square centimetre, calling into question the notion that a "rolling stone gathers no moss"! On one date in 1985 (16 July) and one date in 1987 (9 September), the total algal cells per square centimetre ($R_s = -0.578$) and chlorophyll *a* levels ($R_s = -0.504$), respectively, were greater on smaller stones. Thus, larger stones had lower periphyton densities than smaller stones, a result also reported by Morin and Peters (1988) for a Quebec stream. Similarly, on two dates in 1986 (3 July and 14 August), there was less detritus per unit stone surface area collected with larger stones than collected with smaller stones ($R_s = -0.677$ and -0.583, respectively). Thus, the selection of larger stones by

TABLE 3. Observed and expected numbers of two instar classes of *Megarcys* on stones of two size classes (square centimetres top surface area), 1985–87. Expected frequencies were calculated on the basis of proportion of total surface area represented by each surface area category.

Year	Stone size class	Early instars				Late instars			
		Observed	Expected	$\chi^2_{1\ df}$	p	Observed	Expected	$\chi^2_{1\ df}$	p
1985	>200	17	17.1	0	>0.99	21	15.8	4.15	<0.05
	<200	11	10.9			5	10.1		
1986	>200	106	133.1	14.50	<0.001	17	12.8	4.83	<0.05
	<200	102	75.9			3	7.2		
1987	>200	2947	3034	8.60	<0.01	8	—	—[a]	—
	<200	1326	1239			0	—		

[a]Sample size too small for χ^2 analysis.

TABLE 4. Observed and expected (in parentheses) numbers of stones in two size classes (square centimetres top surface area), September 1987 to July 1988.

Stone size class	Moved	Did not move
<200	13 (7)	38 (44)
>200	0 (6)	39 (33)

$\chi^2_{1\ df} = 13.1, p < 0.001$

Megarcys did not increase their probability of finding abundant food resources used by their prey.

Likewise, not only did larger stones lack predictably high densities of prey populations, but as found by Clements (1987), on several occasions, densities of prey taxa were negatively associated with substrate surface area (20 June 1985, chironomids, $R_s = -0.587$; 24 July 1986, mayflies, $R_s = -0.507$; 12 August 1987, *Epeorus*, $R_s = -0.355$; 9 September 1987, *Baetis*, $R_s = -0.363$). Only on one date (12 August 1987) for one taxon (*Cinygmula* sp.) was prey density significantly positively correlated with stone surface area ($R_s = 0.442$). Thus, selection of larger stones did not increase *Megarcys*' probability of finding high prey densities. Large stones either had the same or lower prey densities than smaller ones. Interestingly, on two dates the mean size of *Baetis* (20 June 1985, $R_s = 0.470$; 30 July 1985, $R_s = 0.361$) and *Epeorus* individuals (20 June 1985, $R_s = 0.602$) increased with stone size. Since larger mayflies have been shown by Allan and Flecker (1988) to be preferred prey of larger *Megarcys*, this result is consistent with the hypothesis that the abundance of a preferred prey type was occasionally higher on stones of larger size.

(4) Factors Associated with Prey Populations

With rare exceptions, densities of the eight major prey taxa were not significantly correlated with water depth (<4%) or interstitial flow (<2% of correlations were significant). As with *Megarcys*, inspection of plots of prey densities versus those physical variables showed no evidence of more complex relationships (e.g. polynomials). Interstitial flows were chosen as the more appropriate flow variable, since relationships between surface flows and benthic densities (e.g. Feltmate et al. 1986) are not relevant to the microhabitat of benthic invertebrates. In fact, as in earlier studies (Peckarsky and Penton 1990), surface flows were poorly correlated with interstitial flows. However, interstitial flow is probably highly variable, and measurements taken at one spot on the underside of a stone may not be indicative of the precise microhabitat of the species in question. Thus, to adequately determine the relationship between flow and habitat selection by stream insects, measurements must be improved either by using more precise techniques (D. D Hart, unpubl. data) or estimating the turbulence or force of flow prevailing at the stream bottom (Peckarsky et al. 1990).

Densities of prey taxa were not consistently higher on granite or slate substrates. On one date (16 July 1985), *Baetis* was more dense on granite stones, and on two dates (12 August and 9 September), both *Ephemerella* and Simuliidae were more dense on slate stones. *Rhithrogena robusta* Dodds was more abundant on slate substrates on all three dates in 1987 (Mann-Whitney *U* test, $p < 0.05$).

Most surprisingly, grazer densities were not significantly correlated with cells per square centimetre of periphyton on any dates in 1985 and were only correlated with chlorophyll *a* levels on one date in 1987 (12 August, $R_s = 0.399$). Thus, contrary to other reports (e.g. McAuliffe 1984), abundance of grazers was not consistently higher on richer periphyton resource. As suggested by Rasmussen and Downing (1988), the renewal rate of algae may be high enough to compensate for the grazing rate of mayflies resulting in neither a consistent positive nor negative association between grazers and their resources.

On the contrary, detritus biomass was consistently positively associated with shredder (largely *Zapada haysi* [Ricker]) densities on all three dates in 1985 ($R_s = 0.745$, 0.523, and 0.640, respectively) and 1986 ($R_s = 0.433$, 0.474, and 0.521, respectively). Thus, shredders did aggregate predictably where their food resources were more abundant, which is consistent with Peckarsky's (1980) findings and with studies conducted in streams where the detritus resource was similarly in short supply (Cowan and Oswood 1984).

Interestingly, densities of prey taxa were often significantly positively autocorrelated on stones sampled at two dates at 2- to 3-wk intervals. In 1985, densities of *Baetis* ($R_s = 0.536$) and *Cinygmula* ($R_s = 0.365$) were autocorrelated between 16 and 30 June, and in 1986, *Epeorus* ($R_s = 0.360$), *Ephemerella* ($R_s = 0.411$), and Chironomidae ($R_s = 0.582$) were autocorrelated between 3 and 24 July and the latter two taxa ($R_s = 0.450$ and 0.393, respectively) between 24 July and 14 August. This result suggests that the same stones replaced in the same spots in the stream were recolonized at similar densities by the same taxa after a disturbance removed all the fauna.

Finally, association analysis among the five most abundant taxa of grazers (mayflies) revealed only one significant negative association (<1% of all correlations were significant). In contrast, 30% of all correlations between pairs of mayfly spe-

TABLE 5. Significant Spearman rank correlation coefficients (R_s) for associations among the five most common grazers in the East River ($R_{s0.05} = 0.355$ and $R_{s0.01} = 0.456$, $n = 30$). The nine values for each interspecific association analysis are given by date as indicated in the first column. — = no *Cinygmula* present in samples.

Date				*Cinygmula*			*Epeorus*			*Ephemerella*			*Rhithrogena*		
1985	1986	1987													
20 June	3 July	12 Aug.	*Baetis*	0.530	0.383			0.440		0.488		0.655			
16 July	24 July	26 Aug.							0.473		0.614			0.359	
30 July	14 Aug.	9 Sept.				—	0.434	0.413	0.539		0.459	0.441			
			Cinygmula				0.547	0.529		0.420		0.367	0.511		
							0.401						0.554		
									—			—			—
			Epeorus												
															−0.540
			Ephemerella												
														0.402	0.465
														0.547	0.484

cies were significantly positive (Table 5). Most of those were due to significant overlap in the distribution of *Baetis* and the other grazer species. This result suggests that stones that are favorable for one grazer taxon are often favorable for others and that, as observed in earlier studies (Peckarsky 1986), rarely (or never) do different species of grazers occupy distinctly different stones.

(Readers interested in more detail on nonsignificant correlations or scatter plots of associated variables may correspond directly with the author.)

Discussion

The descriptive data reported here revealed very few consistent patterns to indicate that predatory stoneflies used physical habitat cues to select habitats in which to forage for prey. The only clear pattern observed was that late-instar predatory *Megarcys* inhabited disproportionately larger stones of those available in the substrate of the East River whereas early instars preferred smaller stones. Contrary to previous reports, there were no clear patterns of stonefly density with surface (Feltmate et al. 1986) or interstitial flows (Peckarsky et al. 1990), nor was water depth related to predator densities. Thus, stone size was the only physical cue among those measured consistently involved in habitat selection by predatory stoneflies.

Larger stones did not have higher densities of prey populations, nor of the resources of prey (periphyton or detritus). Thus, substrate selection by predatory stoneflies apparently did not increase their probability of finding rich prey resource levels. However, large substrates were significantly more stable, with very low probability of displacement during periods of high runoff. These data suggest that large stones make better refuges from trout predation by virtue of lower probability of being disturbed (Feltmate et al. 1986). Other studies have shown that substrate stability is a factor affecting densities of benthic invertebrates (Rounick and Winterbourn 1982; Vannote and Minshall 1982; Robinson and Minshall 1986; Malmqvist and Otto 1987) or argued that the importance of substrate as a refuge from predation may be the selection pressure leading to observed preferences (Brusven and Rose 1981; Hildrew et al. 1980; Flecker and Allan 1984). Large substrates may also offer a more attractive oxygen regime (higher interstitial flows), a speculation that needs to be rigorously tested, or the most suitable size of interstitial spaces increasing their carrying capacity for large predatory stoneflies (Feltmate and Pointing 1986).

The overall rarity of significant associations between benthic densities and physical factors is, perhaps, not surprising when viewed in the context of the complexity of interactions occurring among the various components of the stream ecosystem. First, predators are apparently persistent enough in their prey search activities to prevent negative associations with prey. Yet prey taxa are sufficiently mobile to avoid positive associations with predators (Rasmussen and Downing 1988; Formanowicz and Bobka 1989). Second, both predator and prey behaviors may involve avoidance of competitors, which will also affect their relative distribution patterns (Walde and Davies 1984; Peckarsky and Penton 1985; Peckarsky 1991; P. R Ode, Cornell University, unpubl. data). Third, prey taxa may be tracking their resources (e.g. shredders) or showing a "behavioral response race" with their resources (e.g. grazers) similar to that observed with predators and prey (Sih 1984).

Finally, all those biological interactions are overlain on a template of physical parameters that set some tolerance limits and thus affect habitat selection by both predators and prey. It is no wonder one does not observe more distinct, consistent patterns of relationships between pairs of all these interacting variables taken in the context of the natural stream environment. To do so necessitates the design of controlled experiments to look at pairs of variables in isolation from all the myriad of potentially confounding factors. Unfortunately, such tests usually alter the natural conditions to some extent and can rarely be done on very large spatial or temporal scales. The descriptive approach used in this study, then, has provided hypotheses that can be tested experimentally on potential causation of observed patterns, or lack thereof. The hypothesis suggested by observed patterns (consistent with data reported by Feltmate and Williams 1989) is that habitat selection by predatory stoneflies evolved as a response to pressure from their own predators rather than as an adaptation for efficient acquisition.

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References

ALLAN, J. D. 1982. Feeding habits and prey consumption of three setipalpian stoneflies (Plecoptera) in a mountain stream. Ecology 63: 26–34.

ALLAN, J. D., AND A. S. FLECKER. 1988. Preference in stoneflies: a comparative analysis of prey vulnerability. Oecologia 76: 496–503.

BRUSVEN, M. A., AND S. T. ROSE. 1981. Influence of substrate composition and suspended sediment on insect predation by the torrent sculpin, *Cottus rhotheus*. Can. J. Fish. Aquat. Sci. 38: 1444–1448.

CLEMENTS, W. H. 1987. The effect of rock surface area on distribution and abundance of stream insects. J. Freshwater Ecol. 4: 83–92.

COWAN, C. A., AND M. W. OSWOOD. 1984. Spatial and seasonal associations of benthic macroinvertebrates and detritus in an Alaskan subarctic stream. Polar Biol. 3: 211–215.

COWAN, C. A., AND B. L. PECKARSKY. 1990. Grazing rates of a lotic mayfly as determined by gut fluorescence. J. N. Am. Benthol. Soc. 9: 368–378.

CULP, J. M., S. J. WALDE, AND R. W. DAVIES. 1983. Relative importance of substrate particle size and detritus to stream benthic macroinvertebrate microdistribution. Can. J. Fish. Aquat. Sci. 40: 1568–1574.

FELTMATE, B. W., R. L. BAKER, AND P. J. POINTING. 1986. Distribution of the stonefly nymph *Paragnetina media* (Plecoptera: Perlidae): influence of prey, predators, current speed, and substrate composition. Can. J. Fish. Aquat. Sci. 43: 1582–1587.

FELTMATE, B. W., AND P. J. POINTING. 1986. Effect of habitable space on substrate selection by *Paragnetina media* (Walker) (Plecoptera: Perlidae). Hydrobiologia 131: 277–280.

FELTMATE, B. W., AND D. D WILLIAMS. 1989. Influence of rainbow trout (*Oncorhynchus mykiss*) on density and feeding behavior of a perlid stonefly. Can. J. Fish. Aquat. Sci. 46: 1575–1580.

FLECKER, A. S., AND J. D. ALLAN. 1984. The importance of predation, substrate and spatial refugia in determining lotic insect distributions. Oecologia 44: 306–313.

FORMANOWICZ, D. R. JR., AND M. S. BOBKA. 1989. Predation risk and microhabitat preference: an experimental study of the behavioral responses of prey and predators. Am. Midl. Nat. 121: 379–386.

HAMALA, J. A., S. W. DUNCAN, AND D. W. BLINN. 1981. A portable pump sampler for lotic periphyton. Hydrobiologia 80: 181–191.

HART, D. D. 1979. Association analysis, species interactions, and the structure of benthic invertebrate communities. Can. Spec. Publ. Fish. Aquat. Sci. 43: 63–71.

HILDREW, A. G., C. R. TOWNSEND, AND J. HENDERSON. 1980. Interactions between larval size, microdistribution and substrate in the stoneflies of an iron-rich stream. Oikos 35: 387–396.

MALMQVIST, B., AND C. OTTO. 1987. The influence of substrate stability on the composition of stream benthos: an experimental study. Oikos 48: 33–38.

MALMQVIST, B., AND P. SJÖSTRÖM. 1984. The microdistribution of some lotic insect predators in relation to their prey and to abiotic factors. Freshwater Biol. 14: 649–656.

MAYER, M. S., AND G. E. LIKENS. 1987. The importance of algae in a shaded headwater stream as a food for an abundant caddisfly. J. N. Am. Benthol. Soc. 6: 262–269.

MCAULIFFE, J. R. 1984. Competition for space, disturbance, and the structure of a benthic stream community. Ecology 65: 894–908.

MINSHALL, G. W. 1984. Aquatic insect–substrate relationships, p. 385–400. *In* V. H. Resh and D. M. Rosenberg [ed.] Aquatic insect ecology. Praeger, New York, NY.

MORIN, A., AND R. H. PETERS. 1988. Effects of microhabitat features, seston quality, and periphyton on abundance of overwintering black fly larvae in southern Quebec. Limnol. Oceanogr. 33: 431–446.

PECKARSKY, B. L. 1980. Influence of detritus upon colonization of stream invertebrates. Can. J. Fish. Aquat. Sci. 37: 957–963.

1986. Colonization of natural substrates by stream benthos. Can. J. Fish. Aquat. Sci. 43: 700–709.

1988. Why predaceous stoneflies do not aggregate with their prey. Verh. Int. Ver. Limnol. 23: 2135–2140.

1991. Mechanisms of behavioral interference between stream-dwelling predatory stonefly nymphs. Oecologia. 85: 521–529.

PECKARSKY, B. L., AND S. I. DODSON. 1980. An experimental analysis of biological factors contributing to stream community structure. Ecology 61: 1283–1290.

PECKARSKY, B. L., S. C. HORN, AND B. STATZNER. 1990. Stonefly predation along a hydraulic gradient: a field test of the harsh-benign hypothesis. Freshwater Biol. 24: 181–191.

PECKARSKY, B. L., AND M. A. PENTON. 1985. Is predaceous stonefly behavior affected by competition? Ecology 66: 1718–1728.

1990. Effects of enclosures on stream microhabitat and invertebrate community structure. J. N. Am. Benthol. Soc. 9: 249–261.

RASMUSSEN, J. B., AND J. B. DOWNING. 1988. The spatial response of chironomid larvae to the predator leech *Nephelopsis obscura*. Am. Nat. 131: 14–21.

REICE, S. R. 1980. The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. Ecology 61: 580–590.

ROBINSON, C. T., AND G. W. MINSHALL. 1986. Effects of disturbance frequency on stream benthic community structure in relation to canopy cover and season. J. N. Am. Benthol. Soc. 5: 237–248.

ROUNICK, J. S., AND M. J. WINTERBOURN. 1982. Benthic faunas of forested streams and suggestions for their management. N.Z. J. Ecol. 5: 140–150.

SIH, A. 1984. The behavioral response race between predator and prey. Am. Nat. 123: 143–150.

TAYLOR, L. R., I. P. WOIWOD, AND J. N. PERRY. 1978. The density dependence of spatial behaviour and the rarity of randomness. J. Anim. Ecol. 47: 383–406.

VANNOTE, R. L., AND G. W. MINSHALL. 1982. Fluvial processes and local lithology controlling abundance, structure and composition of mussel beds. Proc. Natl. Acad. Sci. USA 79: 4103–4107.

WALDE, S. J. 1985. Invertebrate predator–prey interactions and stream community structure. Ph.D. dissertation, University of Calgary, Calgary, Alta. 233 p.

WALDE, S. J., AND R. W. DAVIES. 1984. The effect of intraspecific interference on *Kogotus nonus* (Plecoptera) foraging behaviour. Can. J. Zool. 62: 2221–2226.

WILLIAMS, D. D. 1980. Some relationships between stream benthos and substrate heterogeneity. Limnol. Oceanogr. 25: 166–171.

Mating and Multiple Clutches in Australian *Boeckella* and *Calamoecia* (Copepoda: Calanoida)

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Experiments were performed with Australian centropagid calanoid copepods of *Boeckella* and *Calamoecia* to test the hypotheses that species in these genera are capable of producing multiple clutches with one insemination and that successful mating occurs at all stages of the female's ovigerous cycle. The second hypothesis was also tested by an examination of natural populations; spermatophore placement on gravid and nongravid females was scored. Both hypotheses were confirmed. Egg development times and patterns of resting egg hatching were examined under laboratory conditions for up to 100 d. These characteristics and patterns of egg development and resting egg production may result from selection for highly variable and unpredictable environments that existed in Australia before human intervention.

Deux espèces australiennes de copépodes des genres *Boeckella* et *Calamoecia*, du sous-ordre des calanoïdes, ont fait l'objet d'expériences afin de vérifier les hypothèses selon lesquelles ces espèces sont capables de pontes multiples après une seule insémination et que l'insémination peut avoir lieu à tous les stades du cycle reproducteur de la femelle. Cette deuxième hypothèse a aussi été vérifiée par l'examen de populations naturelles. De plus, le site de fixation des spermatophores sur les femelles gravides et non gravides a été noté. Les résultats obtenus étayent les deux hypothèses. Le développement des oeufs et le régime d'éclosion des oeufs d'hiver ont été étudiés en laboratoire pendant une période allant jusqu'à 100 jours. Ces caractéristiques, ainsi que le régime de développement des oeufs et de production d'oeufs d'hiver, peuvent être le résultat d'un choix d'environnements très variables et incertains qui existaient en Australie avant l'intervention humaine.

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In their study of three New Zealand calanoid copepods in the genus *Boeckella*, Jamieson and Burns (1988) comment (p. 255) that one of three species studied ceased reproduction in the absence of males, while two others did not. This observation is surprising because North American calanoids in the genus *Diaptomus* apparently require copulation before each clutch of eggs is produced (Watras and Haney 1980; Watras 1983). Possibly some species in the genus *Boeckella* and in other centropagid genera are capable of sperm storage.

If they are capable of sperm storge, one can predict that copulation might occur anytime in the female's ovigerous cycle; in *Diaptomus*, copulation usually occurs when females are gravid (bearing ripe oocytes) (Wilson and Parrish 1971; Watras and Haney 1980; Watras 1983; Williamson and Butler 1987; Chow-Fraser and Maly 1988; van Leeuwen 1989).

Both sperm storage and copulation at any stage of the ovigerous cycle could be construed as adaptations for completing a life cycle quickly because the time spent finding a mate between reproductions would be reduced and finding a receptive female would not depend upon her reproductive condition. Also, the critical density required to maintain a population would be reduced, since any male–female encounter can lead to the production of a clutch. Thus, it might be possible to find other life history parameters consistent with adaptations for quick completion of a generation.

In this report I describe experiments designed to confirm or disprove Jamieson and Burns (1988) observation that copulation may not be necessary before each clutch is produced in *Boeckella* (and *Calamoecia* which they did not study). I also describe experiments and observations to test the hypothesis that species copulate at any stage of the female's ovigerous cycle, presuming that sperm storage is possible. Finally, I test the hypothesis that egg development time and pattern of hatching may reflect selection for quick generation time.

Materials and Methods

To determine if multiple clutches could be produced by different *Boeckella* and *Calamoecia* species, nine lakes and ponds near Melbourne were sampled with an 80-μm-mesh Wisconsin tow net between March and July 1989 and living organisms were returned to the laboratory in 2-L containers. The ponds were permanent and usually >2 m in depth, except for one which was temporary and <1 m in depth. Twenty females bearing clutches were isolated in 20 mL of lake or pond water placed in small beakers, and each female was observed five to six times per day for 3 d and two to three times per day over the ensuing 4–7 d. Animals were not fed because egg production is related to nutritional status of calanoids with a 10-d time lag (J. T. Turner, Biology Department, Southeastern Massachusetts University, North Dartmouth, MA 02747, pers. comm.). Hatching of the original clutch and production of new clutches were recorded. Eggs that did not hatch after 10 d were considered to be resting or diapausing eggs; these were set aside in covered glass depressions and observed five to seven times per week. Experiments were performed at room temperature,

TABLE 1. Number of additional fertile clutches produced by females of several centropagid species in the absence of males and the maximum time elapsed before production of the last fertile clutch. Twenty females were observed in each trail unless otherwise noted. Each row represents a trial.

	Additional clutches produced					Maximum time (d) to last fertile clutch
	Fertile					
Species	0	1	2	3	Infertile	
B. minuta	1	2	14	3	1	8.2
	0	13	7	0	3	7.0
	2	19	4	0[a]	0	2.9
	16	4	0	0	3	1.5
B. pseudochelae	14	6	0	0[b]	16	2.1
B. symmetrica	2	7	11	0[b]	7	9.8
	2	18	0	0	0	3.5
	14	6	0	0	3	7.3
	0	1	0	0	0	0.8
B. triarticulata	10	15	0	0[a]	6	1.1
	14	6	0	0	0	7.0
	17	3	0	0	1	1.4
B. fluvialis	0	0	0	0	0	—
C. ampulla	0	0	0	0	0	—
C. lucasi	3	9	8	0	2	4.9
	18	2	0	0	1	4.6

[a] Twenty-five females.

[b] Temporary pond.

approximately 20°C, between March and May but dropping slightly to approximately 18°C from late May to July.

To ascertain whether mating can occur anytime in the ovigerous cycle of females and result in the production of a fertile clutch, two or three female *Boeckella triarticulata* and *Boeckella symmetrica* were isolated with three males of the same species in small Petri plates containing 13–15 mL of pond water filtered through 80-μm mesh. These were observed five to six times per day over a 3-d period; ovigerous condition of the female and presence of an attached spermatophore were noted. Females were considered gravid if dark oocytes were observed in the cephalothorax. In addition, numerous populations, preserved in formaldehyde immediately after collection, from lakes and ponds between Canberra and Adelaide were examined. Fifity females (whenever possible) were scored for ovigerous condition and for the presence of a full or empty spermatophore.

In experiments to check for multiple clutches on one insemination, the time taken from clutch production to hatching (or dropping in the case of resting egg production) was recorded.

Results

Females from at least four *Boeckella* species and one *Calamoecia* species are capable of producing additional fertile clutches from a copulation that must have occurred prior to isolation of the females already bearing clutches (Table 1). Where the same species was tested on more than one occasion, from populations taken from different sources or at different times, there was considerable variability both in the number of additional clutches produced and in the maximum time during which additional clutches were produced. Such differences may reflect either the nutritional state of the females or age of the females, but the capability of producing multiple clutches on one insemination is clearly evident.

TABLE 2. Number of spermatophores attached to females in various states of the ovigerous cycle. Each replicate consisted of two to three females placed in 15 mL of pond water with three males, collected at different times.

	With clutch		Without clutch		
Species	Nongravid	Gravid	Nongravid	Gravid	Undetermined[a]
B. triarticulata					
Replicate 1	8	0	3	4	1
Replicate 2	3	3	6	4	5
B. symmetrica	5	0	0	0	2

[a] Ovigerous state altered between observations.

In laboratory conditions, males copulated with females without regard to the ovigerous state of the female (Table 2). In replicate 1 of *B. triarticulata*, males chose gravid females twice and nongravid females five times when there was a choice. In replicate 2, males chose gravid females four times and nongravid females four times. The *B. symmetrica* males could have chosen gravid females four times.

There is a slight but not significant tendency for spermatophores to be found on gravid females overall (Table 3). Paired comparisons of arcsine-transformed data yielded t values of 1.655 (full spermatophores only) and 1.460 (full and empty spermatophores), both not significant, when comparing percentages attached to gravid females and nongravid females. The percentage of nongravid *B. minuta* and *B. symmetrica* females carrying spermatophores was higher than the percentage of gravid females in these species carrying spermatophores.

Development times of eggs at approximately 20°C varied between 1.44 and 2.38 d (Table 4). Some resting eggs from some clutches hatched spontaneously between 14 and 100 d after recovery from experiments, while eggs from other clutches showed no indication of developing or hatching (Table 5). In those clutches where spontaneous hatching occurred, eggs developed to the point where nauplii could be observed within the shell, and hatching then occurred within days. Eggs were allowed to dry in two depressions for 2 d and were again immersed in deionized water; eggs hatched in one replicate.

Discussion

The production of multiple clutches on one insemination occurs in cyclopoids (Gilbert and Williamson 1983) and in the calanoid *Epischura lacustris* (Chow-Fraser and Maly 1988). Many cyclopoid species are found first after ice thaw (personal observation), and *Epischura lacustris* has peak population densities within a month of ice thaw (Chow-Fraser and Maly 1988). Cyclopoids and *Epischura* thus are opportunists, and the fact that several centropagids have at least some capability of producing several clutches on one insemination suggests that they, too, are opportunistic.

Mating at any time during the ovigerous cycle, coupled with the ability to store sperm, also results in a saving of time because a male can copulate with any female encountered, rather than only a gravid female. This phenomenon is not found in North American diaptomids (Chow-Fraser and Maly 1988; van Leeuwen 1989). Data from van Leeuwen (1989) comparable with those in Table 3 indicate that spermatophores are found on gravid females of four *Diaptomus* species approximately four times as often as they are found on nongravid females in natural conditions. In laboratory experiments, Chow-Fraser and Maly

TABLE 3. Percentage of gravid and nongravid females holding spermatophores (full or empty) in field collections from several areas. The percentage of gravid females is also noted.

Species	Number examined	% gravid		% nongravid		% gravid
		Full	Full and empty	Full	Full and empty	
B. triarticulata	687	14.3	21.4	7.5	14.8	20.4
B. minuta	323	0	0	1.4	2.2	32.2
B. fluvialis	258	6.9	10.3	2.6	3.4	11.2
B. symmetrica	334	1.2	1.2	4.4	8.7	24.6
C. lucasi	565	9.2	11.5	4.2	4.4	15.3
C. ampulla	593	9.4	9.4	4.7	5.2	14.3

TABLE 4. Time in days to hatching of clutches produced by female *B. minuta*, *B. triarticulata*, *B. symmetrica*, and *C. lucasi* in laboratory conditions between March and early May when room temperature remained at 20°C.

Species	Source	Development time (SD)	Number measured
B. minuta	Aura Vale March 22	1.60 (0.27)	26
	April 5	1.48 (0.47)	4
B. triarticulata	New Monash Pond March 23	1.44 (0.32)	14
	April 5	1.71 (0.46)	2
B. symmetrica	Lysterfield road May 17	1.93 (0.48)	14
	Churchill Golf Course May 14	2.38 (0.03)	4
C. lucasi	Snake Gulley April 12	1.91 (0.45)	24

TABLE 5. Time in days to hatching of resting eggs produced by *B. triarticulata* and *C. lucasi*. Three *Boeckella* clutches and six *Calamoecia* clutches were observed (C-2 contained three clutches). Clutches B-3 and C-4 were allowed to dry for 48 h on days 1 and 2. The total number of days observed and the percentage of eggs hatching are also provided.

Egg number	*Boeckella* clutches			*Calamoecia* clutches			
	B-1	B-2	B-3	C-1	C-2	C-3	C-4
1	9		10	22		18	
2	21		17	30		24	
3	23		18	32		26	
4	24		18	72		28	
5	28		20	72		28	
6	28		47	83		36	
7	32						
8	50						
Total days observed	95	47	47	85	62	62	62
% hatching	93	0	30	33	0	100	0

(1988) found that males of *D. minutus* and *D. oregonensis* copulated with approximately 50% of gravid females but with only 10–17% of nongravid females.

I calculated egg development time for *Diaptomus* species at 20°C using Bèlehrádek's equation ($D = a/(t-b)^c$ where a, b, and c are constants and t is time and using values for constants summarized by Herzig (1983). The values ranged from 2.13 to 3.25 d for 15 species of *Diaptomus*, considerably longer than the 1.44–2.38 d found in this study (Table 4). The difference is highly significant ($U_{7,19} = 127$, $P < 0.001$). (The values calculated for data reported by Herzig (1983) for *B. symmetrica* (1.72 d) and *C. lucasi* (1.93 d) correspond with values reported in Table 4). This quick development time is another indication that the Australian centropagids are opportunistic.

Finally, the preliminary data reported on resting egg hatching (Table 5) raises the possibility that there are two types of resting eggs: those that are quiescent but ready to hatch at any time and those that are true diapausing eggs requiring some external stimulus to develop. Such a pattern would be similar to that proposed by Elgmork (1980) for diapause in cyclopoid copepods. Furthermore, this pattern would be suited to a highly unpredictable environment where 100% hatch at any one time could result in catastrophe. Two types of observations on natural populations of centropagids are consistent with the notion that there are two types of resting eggs. First, Green (1974) and Bayly (1962) have reported pulses of nauplii in different New Zealand populations of *Boeckella*, suggesting that some environmental factor is synchronizing hatching of resting eggs. On the other hand, I. A. E. Bayly (Department of Ecology and Evolutionary Biology, Monash University, Clayton, Victoria 3168, unpubl. data) reported numerous instances of occurrence of one to three individuals of *Boeckella* and *Calamoecia* in South Australian lakes and ponds sampled by W. Zeidler, while other lakes and ponds have abundant populations. Such low numbers of individuals in small bodies of water that can generally be sampled thoroughly may reflect sporadic hatching of eggs. These differences may be due to asynchrony in hatching or to lake conditions; further studies should indicate which is correct.

In conclusion, species in the genera *Boeckella* and *Calamoecia* possess several characteristics that promote quick completion of a generation. Whether these characteristics have evolved in response to unpredictable environments that were commonplace before water control measures were constructed or are "preadaptations" for successful invasion is unanswered. It would be interesting to examine characteristics of the three *Diaptomus* species in Australia and the one centropagid species found in North America.

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References

BAYLY, I. A. E. 1962. Ecological studies on New Zealand lacustrine zooplankton with special reference to *Boeckella propinqua* Sars (Copepoda: Calanoida). Aust. J. Mar. Freshwater Res. 13: 143–197.

CHOW-FRASER, P., AND E. J. MALY. 1988. Aspects of mating, reproduction, and cooccurrence in three freshwater calanoid copepods. Freshwater Biol. 19: 95–108.

ELGMORK, K. 1980. Evolutionary aspects of diapause in freshwater copepods, p. 388–410. *In* W. C. Kerkfoot [ed.] Evolutionary ecology of zooplankton communities. University Press of New England, Hanover, NH.

GILBERT, J. J., AND C. E WILLIAMSON. 1983. Sexual dimorphism in zooplankton (Copepoda, Cladocera, and Rotifera). Annu. Rev. Ecol. Syst. 14: 1–33.

GREEN, J. D. 1974. The limnology of a New Zealand reservoir with particular reference to the life histories of the copepods *Boeckella propinqua* Sars and *Mesocyclops leuckarti* Claus. Int. Rev. Gesamten Hydrobiol. 59: 441–487.

HERZIG, A. 1983. The ecological significance of the relationship between temperature and duration of embryonic development in planktonic freshwater copepods. Hydrobiologia 100: 65–91.

JAMIESON, C., AND C. BURNS. 1988. The effects of temperature and food on copepodite development, growth, and reproduction in three species of *Boeckella* (Copepoda: Calanoida). Hydrobiologia 164: 235–257.

VAN LEEUWEN, H. C. 1989. Behavioral changes in male *Diaptomus leptopus* (Copepoda: Calanoida) in response to females conditioned water. M.Sc. thesis, Concordia University, Montreal, Que.

WATRAS, C. J. 1983. Reproductive cycles in diaptomid copepods: effects of temperature, photocycle, and species on reproductive potential. Can. J. Fish. Aquat. Sci. 40: 1607–1613.

WATRAS, C. J., AND J. F. HANEY. 1980. Oscillations in reproductive condition of *Diaptomus leptopus* (Copepoda: Calanoida) and their relation to rates of egg-clutch production. Oecologia 45: 94–103.

WILLIAMSON, C. E., AND N. M. BUTLER. 1987. Temperature, food and mate limitation of copepod reproductive rates: separating the effects of multiple hypotheses. J. Plankton Res. 9: 821–836.

WILSON, D. F., AND K. K PARRISH. 1971. Remating in a planktonic marine calanoid copepod. Mar. Biol. 9: 202–204.

Modeling the Stability of Fish Schools: Exchange of Individual Fish between Schools of Skipjack Tuna (*Katsuwonus pelamis*)

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Hilborn, R. 1991. Modeling the stability of fish schools: exchange of individual fish between schools of skipjack tuna (*Katsuwonus pelamis*). Can. J. Fish. Aquat. Sci. 48: 1081–1091.

Three data sets that involve intensive mark and recapture of schools of skipjack tuna (*Katsuwonus pelamis*) are examined for information about exchange of individuals between schools. A formal exchange model is proposed, and maximum likelihood estimates of the parameters are found. It appears that individuals exchange quite rapidly between schools of skipjack; 16–63% of individuals apparently leave a school each day to join other schools. It is possible, however, that what are operationally defined as schools by fishermen consist of smaller more stable subunits.

On a examiné trois ensembles de données recueillies lors d'opérations intensive de marquage et de recapture de thonines à ventre rayé (*Katsuwonus pelamis*) en bancs afin d'avoir des renseignements sur les échanges d'individus entre les bancs. On propose un modèle d'échange établi dans les règles eet l'on donne des estimations de la probabilité maximale des paramètres. Il ressort que les thonines à ventre rayé changent assez rapidement de banc : chaque jour, de 16 à 63 % quittent le banc dont elles font partie pour en rejoindre un autre. Il se peut toutefois que ce que les pêcheurs définissent comme un banc pour les fins de la pêche soit en fait une sous-unité plus petite et plus stable.

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A large portion of the fisheries of the world depend upon harvesting fish aggregated in schools. Nearly all clupeoid fisheries, many salmon fisheries, and surface fisheries for tuna are totally dependent upon school formation to provide vulnerable concentrations of fish. It is therefore surprising that very little is known about the dynamics of fish schools.

The literature on fish schools can be divided into two areas of investigation. One is the determinants of school size. Smith (1970) electronically determined the size of fish schools, and Squire (1978) considered aspects of the shape of the school in relation to size determination. Several others (Breder 1976; Okubo et al. 1977; Anderson 1981) have formulated models of school formation that can be used to explain the size of fish schools.

The second area concerns the stability and integrity of fish schools and seeks to determine if the individual fish physically associated together in a school at a specific time are likely to be together later in the same school. In preliminary investigations, the Inter-American Tropical Tuna Commission (IATTC) (1983) found some data to suggest that skipjack tuna (*Katsuwonus pelamis*) do on occasion tend to stay together based on the occurrence of tagged fish recaptured in the same school as their release. Bayliff (1988) summarized the IATTC work and concluded that complete mixing of schools took place within 3–5 mo after tagging and that generally this mixing had taken place within 1 mo of tagging. Lester et al. (1985) used data on within- and between-school variation in parasites to conclude that complete school mixing took place over a matter of weeks or months. Sharp (1981) has suggested that there may be "core" units within schools that are very cohesive. Cayré (1981) considered the structure of schools and the way that tagging results might be affected by school structure.

The Skipjack Programme of the South Pacific Commission (SPC) conducted a large-scale tagging programme on skipjack tuna in the western tropical Pacific between 1977 and 1980 (Kearney 1983). Of roughly 150 000 skipjack tagged, 6000 were recovered, and a chartered Japanese pole-and-line vessel spent 30 mo sighting over 5000 schools of tuna in the process. Although the principal purpose of the Skipjack Programme was to determine abundance and potential productivity of skipjack in the SPC region, the data accumulated are highly useful for other purposes. In the course of the Skipjack Programme the tagging vessel often recovered fish that had been tagged in the previous few weeks. All fishing was carried out on specific schools; therefore, we know for all tag recoveries the school of release and the school of recapture. Therefore, we can learn a great deal about the exchange of individuals between schools by the analysis of these data.

The purpose of this paper is to determine as much as possible about the exchange of individuals between schools of skipjack tuna using the SPC data base. I will proceed by presenting the recovery data and several alternative hypotheses regarding the behavior of individuals in relation to schools and determining the extent to which an informal survey can discriminate among hypotheses. The remaining hypothesis is then formulated as a mathematical model, and maximum likelihood estimators are derived for its parameters.

Data Available

School Sighting Data

A detailed log was kept onboard the SPC tagging vessel of all schools sighted, giving date, time, latitude, longitude, estimated school size, school species composition, number of

TABLE 1. Logged school sightings and recapture information for visit to Fiji, 10 April 1980 to 27 April 1980.

School No.	No. tagged	No. examined	School size	Date (yr-mo-d)	Time	Lat.	Long.	Recaptures				
1	134	177	8 000	800410	08:00	18°32′S	177°51′E					
2	504	617	8 000	800410	08:30	18°34′S	177°50′E					
3	5	6	2 600	800410	10:00	18°36′S	177°50′E					
4	416	527	2 600	800410	10:30	18°37′S	177°49′E					
5	31	109	2 600	800410	11:45	18°35′S	177°50′E					
6	352	483	8 000	800411	08:00	18°34′S	177°49′E	4-1				
7	12	14	20 000	800411	09:00	18°40′S	177°45′E					
8	1205	1532	8 000	800411	10:00	18°36′S	177°46′E	2-3				
9	505	662	8 000	800411	12:45	18°35′S	177°44′E					
10	7	10	8 000	800411	13:45	18°34′S	177°51′E					
11	49	56	2 600	800411	14:50	18°27′S	177°53′E					
12	992	1153	44 000	800412	08:20	18°35′S	177°49′E	2-1	4-5			
13	8	9	20 000	800412	11:15	18°34′S	177°49′E					
14	84	112	8 000	800413	08:00	18°27′S	177°52′E	8-1				
15	12	16	2 600	800413	09:30	18°33′S	177°48′E	4-1				
16	42	54	2 600	800413	10:30	18°38′S	177°45′E					
17	62	82	8 000	800413	11:20	18°35′S	177°45′E	4-1				
18	526	720	8 000	800413	13:00	18°38′S	177°35′E					
19	168	212	8 000	800413	15:30	18°28′S	177°34′E					
20	10	16	8 000	800414	09:10	18°30′S	177°36′E					
21	409	566	8 000	800414	09:45	18°31′S	177°36′E					
22	1	1	2 600	800414	12:30	18°36′S	177°38′E					
23	6	6	2 600	800414	13:00	18°33′S	177°35′E					
24	29	33	2 600	800414	13:45	18°29′S	177°33′E					
25	24	30	8 000	800415	07:30	18°21′S	177°46′E					
26	543	725	8 000	800415	09:00	18°27′S	177°38′E	21-1				
27	8	8	2 600	800415	13:50	18°25′S	177°41′E					
28	10	12	2 600	800415	14:45	18°24′S	177°48′E					
29	12	12	2 600	800415	15:35	18°21′S	177°52′E					
30	282	394	8 000	800416	11:00	18°12′S	177°29′E					
31	48	82	8 000	800416	12:50	18°09′S	177°25′E					
32	30	55	2 600	800416	13:50	18°09′S	177°21′E	4-1	26-1			
33	110	179	2 600	800416	14:10	18°08′S	177°19′E					
34	106	126	2 600	800417	09:35	17°40′S	176°58′E					
35	27	29	2 600	800418	11:00	16°42′S	177°22′E					
36	196	230	2 600	800418	11:45	16°42′S	177°24′E					
37	130	154	8 000	800418	12:30	16°44′S	177°21′E					
38	1	1	8 000	800419	08:50	16°32′S	177°37′E					
39	1434	1691	20 000	800420	08:15	15°52′S	179°58′W					
40	57	59	2 600	800421	11:20	16°02′S	180°00′W					
41	71	84	2 600	800421	13:20	16°01′S	179°48′E					
42	1	1	2 600	800422	07:10	16°10′S	179°17′E					
43	44	56	2 600	800422	09:45	16°03′S	179°09′E					
44	342	468	8 000	800422	10:50	15°55′S	179°03′E					
45	112	134	2 600	800422	12:00	15°55′S	179°03′E					
46	119	135	2 600	800423	11:00	15°45′S	179°01′E					
47	9	15	2 600	800423	12:00	15°50′S	178°59′E					
48	497	634	2 600	800423	14:00	16°01′S	179°02′E					
49	1	1	2 600	800423	14:50	16°05′S	178°59′E					
50	50	59	2 600	800423	15:45	16°10′S	178°58′E	39-1	40-1			
51	34	35	2 600	800423	17:00	16°09′S	179°03′E					
52	145	168	2 600	800424	07:30	16°11′S	179°02′E	41-1	48-2			
53	617	804	8 000	800424	08:50	16°05′S	178°58′E	39-1	41-7	46-11	48-20	50-2
54	51	55	2 600	800424	11:15	16°08′S	178°58′E					
55	485	559	8 000	800425	15:10	16°12′S	179°03′E	46-1				
56	15	16	8 000	800426	12:00	16°07′S	179°08′E					
57	56	61	20 000	800426	15:45	16°15′S	179°03′E					
58	219	272	2 600	800427	08:15	16°07′S	179°09′E	39-1				
59	191	209	8 000	800427	16:10	15°45′S	179°59′E					

fish tagged, and number of fish taken on board and examined for tags. Estimated school sizes are the weakest data. Schools used in these three areas were classified into one of four categories: small (3–10 metric tons (t)), medium (11–30 t), large (31–70 t), and very large (71–150 t). I used an average weight of 2.5 kg/fish and the midpoint in the range of school size to estimate the number of fish in each size school as 2600, 8000, 20 000, and 44 000, respectively. All field staff I spoke with have cautioned me about the reliability of these estimates. Because many of the subsequent quantitative methods depend upon using school size, the conclusions must be considered in light of the subjectivity of the school size data.

The SPC tagging programme took place in 22 different countries, but only three of these data sets have sufficient recaptures by the SPC vessel to prove useful for our purposes. The characteristics of these three data sets are a large number of fish tagged (greater than 10 000) and concentration of tagging effort in small areas (usually less than 5000 km^2).

Tables show the data for the SPC tagging in Fiji (Table 1) (Kearney 1982), the Marquesas Islands of French Polynesia

TABLE 2. Logged school sightings and recapture information for visit to Marquesas Islands, French Polynesia, 22 December 1979 to 21 January 1980.

School No.	No. tagged	No. examined	School size	Date (yr-mo-d)	Time	Lat.	Long.	Recaptures			
1	0	1	8 000	791222	09:20	10°51′S	141°40′W				
2	59	75	2 600	791222	10:30	10°47′S	141°34′W				
3	0	1	8 000	791222	12:00	10°37′S	141°24′W				
4	69	79	8 000	791222	15:10	10°30′S	141°17′W				
5	75	97	8 000	791222	17:10	10°29′S	141°11′W				
6	205	253	2 600	791223	05:55	09°50′S	139°37′W				
7	8	9	8 000	791223	06:35	09°51′S	139°37′W				
8	134	174	2 600	791223	15:00	09°50′S	139°37′W				
9	25	29	2 600	791223	15:55	09°52′S	139°39′W				
10	215	255	8 000	791223	16:35	09°55′S	139°37′W				
11	39	57	2 600	791223	17:40	10°00′S	139°33′W				
12	406	490	2 600	791223	18:10	10°02′S	139°30′W				
13	51	57	8 000	791224	08:00	08°57′S	140°25′W				
14	243	284	20 000	791224	09:35	08°58′S	140°15′W				
15	86	95	8 000	791224	10:20	09°00′S	140°12′W				
16	384	424	2 600	791224	11:00	09°04′S	140°11′W				
17	657	740	8 000	791224	15:00	09°00′S	140°08′W				
18	22	27	2 600	791224	17:00	09°02′S	140°11′W				
19	32	35	8 000	791225	08:00	09°00′S	140°17′W				
20	47	49	20 000	791225	08:25	09°01′S	140°18′W				
21	0	1	8 000	791225	09:05	09°01′S	140°17′W				
22	54	67	2 600	791225	11:30	09°14′S	139°59′W				
23	0	1	8 000	791225	12:30	09°16′S	139°57′W				
24	845	988	8 000	791225	12:45	09°16′S	139°56′W				
25	128	170	2 600	791225	15:00	09°13′S	140°01′W				
26	185	205	20 000	791225	16:00	09°09′S	140°05′W				
27	510	650	20 000	791225	16:20	09°10′S	140°04′W				
28	25	28	8 000	791225	17:00	09°05′S	140°06′W				
29	237	320	8 000	791226	11:00	09°10′S	140°10′W				
30	41	51	20 000	791226	12:00	09°11′S	140°09′W				
31	70	96	2 600	791226	13:45	09°15′S	140°08′W	27-1			
32	100	146	2 600	791226	15:30	09°14′S	140°00′W				
33	53	71	20 000	791226	16:30	09°07′S	140°07′W				
34	121	166	8 000	791226	17:00	09°05′S	140°09′W				
35	61	87	8 000	791226	17:30	09°01′S	140°08′W				
36	16	19	8 000	791226	17:45	09°00′S	140°08′W				
37	947	1082	44 000	791227	10:50	09°05′S	140°08′W				
38	10	11	2 600	791227	12:45	09°03′S	140°10′W				
39	268	313	8 000	791227	13:00	09°03′S	140°11′W				
40	492	682	20 000	791227	13:30	09°04′S	140°13′W				
41	0	1	8 000	791227	15:40	09°01′S	140°10′W				
42	35	41	8 000	791227	16:50	09°01′S	140°07′W				
43	386	454	8 000	791228	12:15	08°53′S	140°21′W				
44	356	383	44 000	791228	13:00	08°51′S	140°21′W				
45	120	138	8 000	791228	14:15	08°53′S	140°20′W				
46	167	196	8 000	791228	14:30	08°54′S	140°20′W				
47	11	16	2 600	791228	15:00	08°55′S	140°20′W				
48	5	7	8 000	791230	11:20	09°01′S	140°16′W				
49	28	32	20 000	791230	12:30	08°54′S	140°16′W				
50	19	20	8 000	791231	11:40	09°01′S	140°09′W				
51	36	41	8 000	791231	12:15	09°02′S	140°11′W				
52	54	64	20 000	791231	12:35	09°03′S	140°09′W				
53	441	532	20 000	800104	11:15	09°00′S	140°05′W				
54	27	29	20 000	800104	12:15	09°04′S	140°04′W				
55	6	7	44 000	800105	09:45	08°59′S	140°04′W				
56	433	480	20 000	800105	11:00	09°05′S	140°09′W				
57	210	236	20 000	800105	12:30	09°08′S	140°06′W				
58	29	29	8 000	800105	13:30	09°13′S	140°07′W				
59	107	125	20 000	800105	14:00	09′15′S	140°06′W				
60	471	557	8 000	800105	15:00	09°13′S	140°03′W				
61	297	338	8 000	800105	15:30	09°10′S	140°02′W	17-1			
62	697	801	44 000	800106	12:00	08°54′S	140°17′W	17-1			
63	8	8	8 000	800106	15:50	08°53′S	140°19′W				
64	12	13	2 600	800107	12:00	08°53′S	140°19′W				
65	34	36	2 600	800107	13:15	08°56′S	140°21′W				
66	425	481	2 600	800107	14:00	08°54′S	140°19′W	24-1	40-1	60-1	37-2
67	152	172	2 600	800107	15:00	08°53′S	140°16′W	16-1	37-1	62-1	
68	1	1	2 600	800107	15:15	08°52′S	140°17′W				
69	19	20	2 600	800107	16:35	08°58′S	140°15′W	17-1			
70	5	6	20 000	800108	08:30	08°47′S	140°18′W				
71	297	320	20 000	800108	10:30	08°45′S	140°23′W	17-1			

TABLE 2. (*Concluded*)

School No.	No. tagged	No. examined	School size	Date (yr-mo-d)	Time	Lat.	Long.	Recaptures			
72	37	41	8 000	800108	12:30	08°49′S	140°19′W				
73	216	242	8 000	800108	13:30	08°47′S	140°23′W				
74	154	182	8 000	800108	14:20	08°52′S	140°21′W				
75	148	168	2 600	800108	15:50	08°56′S	140°17′W				
76	449	517	44 000	800109	11:50	09°00′S	140°16′W	16-1	37-1	44-1	46-1
77	70	80	20 000	800110	11:15	09°01′S	140°12′W				
78	0	1	8 000	800111	11:30	08°58′S	140°14′W				
79	369	422	20 000	800111	12:15	08°57′S	140°16′W	37-2	44-1		
80	188	213	8 000	800111	13:45	08°56′S	140°18′W	53-1	60-1		
81	101	112	8 000	800111	14:00	08°56′S	140°17′W				
82	107	124	8 000	800111	14:50	08°58′S	140°19′W				
83	703	775	20 000	800112	11:20	08°54′S	140°15′W	37-1	60-1		
84	28	29	20 000	800112	12:45	08°53′S	140°20′W				
85	14	17	8 000	800112	13:50	08°50′S	140°17′W				
86	196	213	8 000	800113	15:00	09°02′S	140°15′W				
87	91	103	8 000	800113	16:00	09°03′S	140°06′W				
88	405	479	8 000	800114	13:00	09°01′S	140°06′W	62-1			
89	311	373	20 000	800114	14:15	09°02′S	140°16′W				
90	53	62	8 000	800114	15:30	09°01′S	140°15′W				
91	122	131	2 600	800115	07:30	09°17′S	140°05′W				
92	220	254	20 000	800115	09:30	09°14′S	140°15′W				
93	3	3	2 600	800115	11:30	09°03′S	140°14′W				
94	155	175	8 000	800115	14:30	09°02′S	139°59′W				
95	0	1	8 000	800116	11:15	09°00′S	139°59′W				
96	18	20	8 000	800116	13:10	08°55′S	139°49′W				
97	98	114	2 600	800116	14:30	09°02′S	139°57′W				
98	902	1007	2 600	800116	16:00	08°53′S	139°56′W	20-1	56-1		
99	789	909	2 600	800117	14:00	08°53′S	139°51′W				
100	16	19	2 600	800118	14:15	08°53′S	140°19′W				
101	30	34	2 600	800118	15:00	08°59′S	140°18′W				
102	100	112	8 000	800118	17:00	08°58′S	140°15′W	17-1	56-1	76-1	90-1
103	156	173	8 000	800119	16:15	08°52′S	139°57′W	53-1			
104	81	87	2 600	800119	17:00	08°49′S	139°57′W	37-1			
105	125	144	2 600	800120	16:30	08°50′S	139°55′W	60-1			
106	98	108	8 000	800120	18:00	08°44′S	140°02′W				
107	232	274	20 000	800121	17:00	10°22′S	141°18′W				

(Table 2) (Gillett and Kearney 1983), and the island of Wallis, a French Territory (Table 3) (Tuna Programme 1984). The recapture information in Tables 1–3 consists of the school number followed by the number of fish marked in that school that were recaptured in the school described on the same line. We can therefore see that it was reasonably common for a tagged fish to be recaptured, but recaptures of more than one or two fish were uncommon. The single school (No. 53) in the Fiji data set, in which large numbers of fish from several schools were recaptured, is the major exception to this.

The Wallis visit took place in a very small area whereas some schools were encountered over wide ranges in the Fiji and Marquesas visits. However, almost all tagging in the Marquesas visit was done within a rather small area, and the Fiji tagging took place in two areas of concentration. Thus I use only the data from the intensely tagged areas for analysis, i.e. all of the data for the Wallis visit and schools 13–106 for the Marquesas visit. The Fiji tagging essentially consists of two data sets; no tagged fish were recovered from the southern data in the north; therefore, I have divided the Fiji data into one set (denoted Fiji-a) which is schools 1–29 and a second set (denoted Fiji-b) which is schools 42–58. All four data sets, therefore, correspond to very intense tagging over 1–4 wk in an area approximately 0.5° latitude and 0.5° longitude on each side. This corresponds to an area of 5000 km^2.

The areas covered (Table 4) were computed by taking the mean latitude and longitude, calculating a radius to the farthest school from this mean location, and calculating the area of a circle with the radius calculated. The area is 2638–4959 km^2 for the first Fiji visit and the Marquesas visit, respectively. The Wallis and Fiji-b visits have intermediate areas.

We can make rough estimates of the number of schools in each area by utilizing the sighting frequency. Kearney (1982), Gillet and Kearney (1983), and Tuna Programme (1984) gave the encounter rates of 0.877 school per hour for Wallis, 0.797 school per hour for Fiji, and 0.762 school per hour for the Marquesas. If we assume that the SPC tagging vessel was travelling at 20 km/h, and schools were visible at 1 km on either side of the ship (note that this is probably an extreme underestimate), then the SPC vessel was searching 40 km^2/h. Dividing the encounter rates by the area searched per hour provides the estimate of school density per square kilometre given in Table 4. We can then multiply this by the area searched to arrive at an estimate of the number of schools in the area of tagging for each visit.

We can make another estimate of the abundance of fish in the area by treating the tagging vessel recoveries as a mark–recapture experiment, a Schnabel census to be specific. Seber (1982) described the standard techniques for the analysis of this type of information. By using the average school size of 10 000 individuals (the observed average rounded to nearest 1000), we can then compute from this method how many schools were in the area (Table 4). Note that the estimated number of fish from the Schnabel census is far higher than the number estimated

TABLE 3. Logged school sightings for visit to Wallis, 6–31 May 1978.

School No.	No. tagged	No. examined	School size	Date (yr-mo-d)	Time	Lat.	Long.	Recaptures	
1	85	126	8 000	780506	08:40	13°27′S	176°07′W		
2	602	882	20 000	780506	09:30	13°26′S	176°02′W		
3	509	813	8 000	780506	12:50	13°21′S	176°02′W		
4	62	74	2 600	780506	15:00	13°22′S	176°03′W		
5	96	142	8 000	780512	07:55	13°25′S	176°11′W		
6	332	426	8 000	780512	11:20	13°07′S	176°07′W		
7	137	208	8 000	780512	12:50	13°08′S	176°13′W		
8	23	28	20 000	780512	15:00	13°21′S	176°15′W		
9	73	83	8 000	780512	16:30	13°25′S	176°14′W		
10	332	407	20 000	780513	09:30	13°28′S	176°13′W		
11	6	8	2 600	780513	12:00	13°14′S	176°16′W		
12	128	195	8 000	780513	12:20	13°13′S	176°15′W	10-1	
13	300	362	8 000	780513	14:50	13°28′S	176°18′W		
14	75	90	8 000	780514	09:10	13°20′S	176°24′W		
15	116	127	8 000	780514	11:10	13°08′S	176°22′W		
16	200	229	20 000	780515	07:10	13°23′S	176°13′W		
17	59	80	8 000	780515	08:00	13°24′S	176°12′W		
18	4	4	2 600	780515	10:00	13°10′S	176°18′W		
19	410	499	8 000	780515	10:35	13°09′S	176°22′W		
20	194	224	8 000	780515	13:00	13°08′S	176°22′W	17-1	
21	10	12	2 600	780515	13:50	13°09′S	176°15′W		
22	5	5	2 600	780515	16:15	13°27′S	176°11′W		
23	263	295	8 000	780516	12:15	13°25′S	176°09′W	5-1	
24	768	886	20 000	780516	13:50	13°30′S	176°05′W	2-1	
25	19	21	8 000	780517	10:30	13°37′S	176°04′W		
26	1034	1161	20 000	780517	11:50	13°29′S	176°07′W		
27	742	836	20 000	780518	08:50	13°31′S	176°05′W	26-3	
28	293	307	8 000	780518	11:05	13°34′S	176°12′W		
29	363	401	8 000	780519	07:35	13°24′S	176°10′W	28-1	
30	194	261	8 000	780519	09:15	13°28′S	176°07′W	24-1	
31	1028	1183	20 000	780519	10:30	13°30′S	176°05′W	29-2	
32	317	381	8 000	780519	11:50	13°31′S	176°08′W	31-1	
33	60	65	8 000	780519	13:20	13°29′S	176°14′W		
34	350	484	8 000	780520	08:50	13°17′S	176°23′W		
35	1565	1725	20 000	780520	10:30	13°15′S	176°20′W		
36	1006	1057	20 000	780520	12:30	13°17′S	176°16′W		
37	417	553	8 000	780521	08:15	13°32′S	176°10′W	36-2	
38	49	59	20 000	780524	07:30	13°29′S	176°09′W	27-1	
39	50	64	2 600	780524	08:50	13°30′S	176°01′W		
40	38	44	2 600	780524	12:00	13°26′S	176°14′W		
41	137	147	2 600	780525	09:45	13°36′S	176°01′W		
42	9	9	20 000	780525	10:50	13°38′S	176°00′W		
43	122	172	8 000	780526	08:15	13°19′S	176°17′W		
44	81	94	20 000	780526	09:45	13°10′S	176°18′W	29-1	31-1
45	27	34	2 600	780527	12:20	13°13′S	176°15′W		
46	140	160	8 000	780529	07:30	14°11′S	177°54′W		
47	308	346	8 000	780529	08:30	14°05′S	177°58′W		
48	38	186	20 000	780529	10:45	14°13′S	178°03′W		
49	337	445	8 000	780531	15:00	13°13′S	174°58′W		

TABLE 4. Estimates of area covered, school density, and number of schools derived from sightings data. Total population size estimate derived from Schnabel census, and estimated number of schools and apparent vulnerability of tagged fish are also shown. No results are presented for the Schnabel census for Fiji-b because of computational difficulties associated with the low likelihood of the recaptures in school 53.

Visit	Area (km^2)	School density (no./km^2)	No. of schools by sightings	Population size estimate (millions)	No. of schools, Schnabel census	Implied vulnerability
Wallis	3981	0.021	84	6.0	600	0.14
Marquesas	4959	0.019	95	4.8	480	0.20
Fiji-a	2638	0.019	51	1.55	155	0.33
Fiji-b	4710	0.019	91	—	—	—

from the sighting data. Several explanations are possible. There would be some movement of fish in and out of the tagging areas during the period of tagging. The school sighting rate may be too low because of the possibility of not seeing schools which are submerged or otherwise difficult to see. However, the use of 1 km visibility on either side of the vessel is almost certainly an underestimate; thus, it is equally likely that we are overestimating the abundance of schools. The estimated number of schools from the Schnabel census depends upon the school size judgements, known to be unreliable. Shown in the far right-hand column of Table 4 is the implied vulnerability of tagged fish, which is the ratio of the number of schools estimated by the mark–recapture data divided into the number of schools estimated by direct sightings.

It seems most likely that the discrepancy between the two approaches is due to reduced vulnerability of tagged fish. We will see that the vulnerability of fish is probably reduced at the time of tagging and this reduced vulnerability could easily last for several days. The comparison of the two methods suggests that they can be reconciled by assuming that tagged fish are only 15–30% as vulnerable as untagged fish. This may be a good estimate, and we will examine it later in light of the analysis of the formal models.

Analysis

Alternative Hypotheses

The simplest model of school stability is one in which there is a gradual decay of individuals away from the school over time. The longer it has been since a group of fish were tagged in the same school, the less likely it is that they would be still together in the same school. This hypothesis is consistent with a number of possible mechanisms: individuals could have a certain probability of leaving the school per unit time, much like radioactive decay, or the schools could disaggregate each night and reform in the morning. The probability that an individual would reform with its school of the previous day would be another form of decay rate. In this simple hypothesis, we would assume that if an individual was not associated with the school in which it was tagged, then it would have an equal probability of being associated with the other schools in the vicinity. Obvious extensions of this hypothesis would consider the probability of associating with another school to be related to the distance between them. This hypothesis can therefore encompass completely rigid schools (decay rate 0.0), or totally random associations in which schools completely dissolve each day (decay rate very high).

An alternative hypothesis is that schools exchange individuals when they are in close proximity to one another, and perhaps schools merge together and break apart. Under this hypothesis, we can again have very rigid schools where the proportion exchanged when two schools come together is very small, or the frequency of encounter between schools is low, and we could have highly fluid schools which exchange a large portion of individuals when schools come together.

The major difference between the two hypotheses is in the likelihood that several individuals from separate schools would be found in association with one another. Under the first hypothesis it should be quite unlikely ever to find more than one or two individuals tagged in different schools recovered in the same school. However, under the second hypothesis, two schools which exchanged significant proportions of individuals could then have a large number of tagged fish from two separate schools. Both of these general conclusions depend upon the actual exchange rates, number tagged, etc., and thus provide the motivation for the more formal model presented later.

Examination of the data in Tables 1–3 provides some insight into the relative likelihood of these two hypotheses. It is not uncommon to find individuals tagged from several schools together in the same school. However, with the exception of school 53 in the Fiji data (Table 1), we never find large numbers of individuals tagged in separate schools recovered in the same school. In this one instance, it is possible that this school was in fact tagged several times. Analysis of the search tracks of the SPC vessel, described above, suggests that it is quite likely that the same school could have been fished several times. However, when we look at school 53 from Fiji, we find that while it is possible that schools 41, 46, 48, and 53 might in fact be the same school, we would have expected that when tagging school 46, we would have recovered some tagged fish from school 41, and similarly when tagging school 48, we should have recovered tagged fish from schools 41 and 46.

Insight into the loss rate from schools can be gained by examining some of the cases where several individuals tagged in the same school were recaptured in the same school. In the Fiji data (Table 1), five individuals from school 4 were recaptured in school 12, within a few miles of the location where school 4 was tagged. It would seem likely that school 12 is therefore the same school as school 4. However, school 4 was estimated to be between 1 and 10 t, or to contain an expected average of 2500 individuals. Four hundred and sixteen skipjack were tagged in school 4, which is 16%. In school 12, 1153 individual skipjack were taken on board and examined (either tagged or retained as specimens); of these, only 5 bore tags from school 4. School 12 was classified as a very large school and has an expected 44 000 individuals. If school 4 had amalgamated with another school to form part of school 12, then 416/44 000 or 0.95% of individuals of school 12 would be tagged from school 4. We would therefore expect 10.9 recaptures from school 4 in school 12, not drastically different from the 5 observed, especially when the subjectivity of the school size data mentioned earlier is considered.

It is more difficult to interpret the recaptures in school 8 of three fish tagged in school 2. Both schools 2 and 8 were rated as medium schools, with an expected average of 8000 individuals. Therefore, it might seem reasonable to assume that these are the same school. However, 504 of 8000 fish were tagged in school 2 (6.3%). If there is no loss from the school in the 1 d that elapsed between the encounters with schools 2 and 8, then of the 1532 individuals in school 8, 96.5 of them should have been tagged.

There are at least three ways to explain this result. Either schools break up rapidly and school 8 was the same as school 2 but had lost most of its tagged individuals, or school 8 was a different school that exchanged individuals with school 2. Alternatively, schools 2 and 8 were identical schools, but the tagged individuals are less likely to be captured than untagged fish or there is high tagging mortality. Data presented later show that there is probably a temporary reduction in vulnerability of tagged fish.

The three data sets in Tables 1–3 show that, with the exception of school 53 in the Fiji data, there were no large recaptures of many individuals tagged in the same school. The pattern is rather one of many individual recaptures of one or two fish, often fish tagged in different schools. This picture

TABLE 5. Expected and observed number of fish immediately recaptured within the same school in which they were tagged. The expected number is based on uniform mixing of the school and equal probability of tagged and untagged fish.

Country	Expected (E)	Observed (O)	Proportion O/E
Fiji	411	10	0.0243
Marquesas	617	1	0.0016
Wallis	293	33	0.1126
Total	1321	44	0.033

would therefore seem consistent with our first model, of a reasonably rapid exchange of fish between schools, and a high number of schools present so that the chances of encountering a previous school would be low.

Probability of Biting

A problem common with many tagging studies is the unknown effect of tagging on future survival and behavior of tagged fish. It has generally been assumed that tagged fish will behave identically to untagged fish, an assumption that is difficult to verify in practice. The artisanal fishermen of Fresh Polynesia believe that it is very bad to lose a fish from a hook because the escaping fish will dive deep and "drag" some of the school with it, perhaps frightening them. The fact that the SPC vessel would occasionally recapture tagged fish within minutes after tagging is inconsistent with this belief.

We can use the data from Tables 1–3 and the information on immediate recaptures to determine if tagged fish are equally likely to bite after being tagged. If we view the school as completely mixed (a questionable assumption discussed later), so that once a fish is tagged it has the same probability of being recaptured as an untagged fish in the same school, then for a school in which T_i fish are tagged, and the school consists of N_i fish, the number of expected recaptures while tagging the school (I_i) will be

$$(1) \quad I_i = \frac{\frac{T_i}{2}(T_i - 1)}{N_i}.$$

If we had 100 individuals in a school and tagged 5 of them, then the expected number of recaptures would be the sum of probabilities of recapturing a fish on the second to fifth fish captured or 1/100 + 2/100 + 3/100 + 4/100. Equation 1 is simply an expression for this sum.

We can then use the number tagged and school sizes from Tables 1–3 to determine the expected number of fish immediately recaptured. The expected number of immediate recaptures and the actual number of recaptures are shown in Table 5. Fish that are tagged appear to be less likely to bite in the hour or two after being tagged; on average they have only 3% of the probability of biting as an untagged fish. If school size was consistently underestimated, then the probability of biting after tagging would be higher. The mechanism for reduced probability of capture may also be that individuals leave the school after being tagged; they simply flee the scene of their capture. This analysis also assumes homogeneity of the tagged and untagged population. Frequently the tagging vessel moved through a school as it was tagging, so that if individuals did not move once they were returned to the water after tagging the vessel would have often moved past them. Given these limitations, we cannot be too sure that the apparent reduced probability of biting after tagging is as pronounced as it appears on first analysis.

Formal Models

The casual examination of the three data sets discussed above cannot lead to any definitive conclusions about relative probabilities of alternative hypotheses. Formal models are required to properly analyze these data. We can formulate one hypothesis as follows.

Assume that the area covered by any specific visit of the SPC tagging vessel contains a fixed number of schools of skipjack (S), that the size of each school i is N_i, and that there is a rate at which fish leave schools (d) and once they have left a school they reassociate instantly and at random with another school in the area. Assume that the probability of associating with any other school is the same for all schools, regardless of their size.

In the course of a country visit, the SPC tagging vessel will encounter and tag from a number of schools (n), and for each school the date (D_i), the size (N_i), the number of tagged fish released (T_i), the number of fish examined (E_i), and the recaptures of fish tagged in school i (R_{ji}) are all recorded. Further, assume that once a fish is tagged it has a vulnerability (V) to the fishing gear in the future (with $V < 1$). The parameter V can be thought of as either the reduced likelihood of biting a hook, or one minus tagging mortality, or a surrogate for emigration as discussed later.

The number of tagged fish remaining in school i t days after tagging (L_{it}) will be

$$(2) \quad L_{it} = T_i e^{-dt}.$$

The number of fish that are lost from the school after t days will therefore be $T_i - L_{it}$. The number of fish marked in the ith school present in the jth school at the time the jth school is tagged (t days after tagging in the ith school) will therefore be

$$(3) \quad M_{ijk} = \begin{cases} \dfrac{T_i(1-e^{-dt})}{S}, & \textit{if } i \neq \mathrm{k} \\ L_{it'} & \text{if } i = k \end{cases}$$

where k represents the true identity of the school. That is, if school j is the same school as school 1, then $k = 1$. For convenience we can consider k to range between 1 and $j - 1$, with the value of $k = j$ representing any school which has not previously been tagged. There are therefore j possible hypotheses about the true identity of the school.

The conditions in equation 3 above recognize that we may tag a specific school more than once; for instance, schools 4 and 12 in the Fiji-a data set may have been the same school. It also assumes that a fish that has left school i has an equal probability of joining any other school, regardless of its size, and no probability of rejoining the school it left.

The number of recaptures can be expected to be binomially distributed, depending upon the proportion of fish marked in the school and the number examined. The proportion of fish in school j that were marked in school i (p^*_{ijk},) will be M_{ijk}/N_j. Since we are assuming that marked fish are less likely to bite a hook than unmarked fish, the probability that a fish which bites a hook during tagging school j was tagged in school i (p_{ijk}) will be $p^*_{ijk} \times V$. The probability of observing R_{ijk} recaptures of fish tagged in school i when examining E_j fish in school j, given the true identity of the school is k, is therefore

$$(4) \quad \mathrm{pr}(R_{ijk}) = \frac{E_j!}{R_{ijk}!(E_j - R_{ijk})!} p_{ijk}{}^{R_{ijk}} (1 - p_{ijk})^{(E_j - R_{ijk})}.$$

Table 6. Example calculations for Fiji-a visit, recoveries from school No. 6. School size $N_6 = 8000$ fish; number of fish examined in school 6, $E_6 = 483$. School 6 was tagged after schools 1–5. All calculations assume that $d = 0.1$, $S = 100$, and $V = 1.0$.

No. of tags left in each school and number lost

	School 1	School 2	School 3	School 4	School 5
Tags recovered R_{i6}	0	0	0	1	0
No. tagged T_i	134	504	5	416	31
Tags left L_{i1}	121.248	456.038	4.524	376.412	28.050
Tags lost	12.752	47.962	0.476	39.588	2.950

Expected no. of tags present in school 6, M_{i6k}

	From school No. (i)				
If this was school:	1	2	3	4	5
$k=1$	121.2482	0.4845	0.0048	0.3999	0.0298
$k=2$	0.1288	456.0381	0.0048	0.3999	0.0298
$k=3$	0.1288	0.4845	4.5242	0.3999	0.0298
$k=4$	0.1288	0.4845	0.0048	376.4124	0.0298
$k=5$	0.1288	0.4845	0.0048	0.3999	28.0500
New school: $k=6$	0.1288	0.4845	0.0048	0.3999	0.0298

Proportion of fished tagged in school 6, $p_{i6k} = M_{i6k}/N_6$

	From school No. (i)				
If this was school:	1	2	3	4	5
$k=1$	0.152×10^{-1}	0.606×10^{-4}	0.601×10^{-6}	0.500×10^{-4}	0.372×10^{-5}
$k=2$	0.161×10^{-4}	0.570×10^{-1}	0.601×10^{-6}	0.500×10^{-4}	0.372×10^{-5}
$k=3$	0.161×10^{-4}	0.606×10^{-4}	0.566×10^{-3}	0.500×10^{-4}	0.372×10^{-5}
$k=4$	0.161×10^{-4}	0.606×10^{-4}	0.601×10^{-6}	0.471×10^{-1}	0.372×10^{-5}
$k=5$	0.161×10^{-4}	0.606×10^{-4}	0.601×10^{-6}	0.500×10^{-4}	0.351×10^{-2}
New school: $k=6$	0.161×10^{-4}	0.606×10^{-4}	0.601×10^{-6}	0.500×10^{-4}	0.372×10^{-5}

Binomial probability of observing the actual recoveries
given the expected proportion marked pr(R_{i6k})

	Recoveries from school No. (i)				
If this was school:	1	2	3	4	5
$k=1$	0.626×10^{-3}	0.971	1.0	0.0236	0.998
$k=2$	0.992	0.488×10^{-12}	1.0	0.0236	0.998
$k=3$	0.992	0.971	0.761	0.0236	0.998
$k=4$	0.992	0.971	1.0	0.185×10^{-8}	0.998
$k=5$	0.992	0.971	1.0	0.0236	0.183
New school: $k=6$	0.992	0.971	1.0	0.0236	0.998

Probabilities of observing the recovery pattern for marks from all five previous schools

If this was school:	Prob. of recoveries	Probability it was this school pr (k)	Product
1	1.429477×10^{-5}	0.01	1.429477×10^{-7}
2	1.137961×10^{-14}	0.01	1.137961×10^{-16}
3	1.725031×10^{-2}	0.01	1.725031×10^{-4}
4	1.782363×10^{-9}	0.01	1.782363×10^{-11}
5	4.162285×10^{-3}	0.01	4.162285×10^{-5}
6	2.266380×10^{-2}	0.95	0.0215
		Total probability	0.0217

Note that M_{ijk} will depend upon the number of days elapsed between tagging of fish in school i and the subsequent tagging in school j.

If we knew the number of schools in the area (S) and the rate at which fish leave the school they are in (d), we could then use equation 3 to determine how many individuals marked in each school we would expect to be present in any other school, and if we knew the vulnerability of previously marked fish, we could then use equation 4 to calculate the probability of recapturing any specific number of fish tagged in i while fishing and tagging school j. There are two probabilities, however: one if school j is the same as school i (the second case of equation 3) and the other that school j is a different school from school i (the first case of equation 3). The probability that school i and j are the same is simply $1/S$.

Table 6 shows a detailed worked example, using the data from schools 1–6 from the Fiji-a visit. Schools 1–5 had 134, 504, 5, 416, and 31 fish tagged, respectively, all on the 4 of

October 1980. The next day, 483 fish in school 6 were examined for tags, and there was one tag from school 4. Given that 1 d had elapsed, the top section of Table 6 gives the number of fish tagged, the number that would be expected to be left, and the number lost to other schools assuming $d = 0.1$. Assuming that there are 100 schools in the area ($S = 100$), the second section of Table 6 shows how many tagged fish from each school (columns) would be expected to be present in school 6 (M_{i6k}), if school 6 was any of the previous schools (first five rows, $k = 1$–5), or one of the 95 schools that had not been previously encountered ($k = 6$).

Thus if school 6 was the same school as school 1, we would expect many tagged fish from school 1 to be present, but if school 6 was a totally new school, we could expect very few tagged fish from school 1 (or any other tagged fish for that matter) to be present. Thus the nondiagonal elements in this portion of the table are large, while all other elements are small.

The third portion of the table shows the proportion (p_{i6k}) of fish in school 6 that would be expected to be marked by tagged fish of other schools (columns) given that school 6 was one of the previous schools, or a new one (rows). Again, the diagonal elements are reasonably big, while the nondiagonal elements are small.

The fourth section of Table 6 shows the binomial probabilities (pr{R_{ijk}} calculated from equation 4) of observing the recoveries from each school, if school 6 was actually one of the previous schools or a new one. For this example we are assuming that tagged fish have an equal probability of biting a hook ($V = 1.0$). In this case the diagonal elements are quite small. This simply says that if this was really a school previously encountered, it is most unlikely that no tagged fish would have been recovered. This is also true for school 4, even though one tag was recovered. If school 6 was the same school as school 4, then there should have been 4.7% of the fish with tags from school 4, and thus numerous recaptures would have been expected. The fact that only one tag was recovered makes it unlikely that schools 4 and 6 were the same school. Since only five fish were tagged in school 3, it is quite possible that schools 3 and 6 are identical. This is the major exception in the diagonal element of this part of the table.

The final section of Table 6 shows in the first column the product of the binomial probabilities over all tag recoveries pr ($R_{.6k}$) = $\Pi^5_{i=1}$pr(R_{i6k}). The second column gives the probability that it is the school in question ($1/S$) or a new school ($(S-j+1)/S$), and the third column is the product of the first two columns.

The total likelihood of observing the tag recovery pattern over all possibilities of the identity of school 6 is pr($R_{.6}$), given the assumed values of d, S, and V. Because these are not normalized, they are best thought of as likelihoods rather than probabilities.

TABLE 7. Parameters estimated from school data using maximum likelihood method (*parameter constrained to that value).

Visit	School breakup rate (d)	No. of schools (S)	Vulnerability of tagged fish (V)
Wallis	1.0	263	0.265
Wallis	1.0	291	0.300*
Marquesas	0.182	717	0.996
Marquesas	0.300	365	0.300*
Fiji-a	0.450	249	1.000
Fiji-a	0.355	103	0.300*

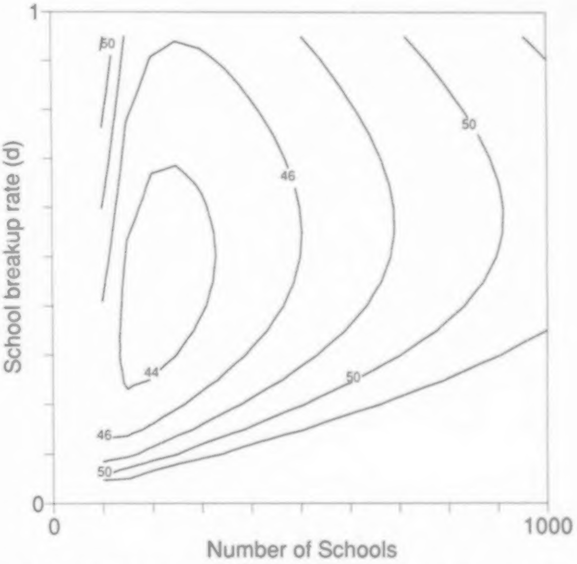

FIG. 1. Negative log-likelihood contours for Fiji-a data set with V fixed as 1.0.

Parameter Estimation

We now need to formulate a method of estimating the three parameters d, S, and V from the data available. The example in Table 6 shows how we can calculate the likelihood of the observations in a single school given particular parameter values for d, S, and V. Continuing with the notation pr(R_{ijk}) as the probability of observing R recaptures from the ith school in the jth school is the same as the kth school, denote

$$(5)\quad \mathrm{pr}\,(R_{.jk}) = \prod_{i=1}^{j-1} \mathrm{pr}(R_{ijk})$$

as the probability of observing the number of recaptures from all previously tagged schools in the jth school given school identity k. The probability of observing the recaptures found in the jth school over all possible school identities (k's) can be calculated as

$$(6)\quad \mathrm{pr}\,(R_{.j.}) = \sum_{k=1}^{j} \mathrm{pr}(k)\mathrm{pr}(R_{.jk})$$

where pr(k) is the probability that the school is the kth school which is $1/S$ for all k's except $k = j$, and for $k = j$, pr(k) = $(S - (j - 1))/S$.

Finally the probability of observing the entire sequence of recaptures for an entire visit is the product of the probabilities of observing the recaptures in each school from 2 to n, or

$$(7)\quad \mathrm{pr}\,(R_{...}) = \prod_{j=2}^{n} \mathrm{pr}(R_{.j.}).$$

This equation provides the likelihood function which we can seek to maximize by searching over possible values of the three parameters d, S, and V.

Results

Table 7 shows the parameters estimated using the model on the data sets available. The analysis of the Wallis data indicates rapid breakup of the schools, an estimated 263 schools in the tagging area, and the probability of a marked fish biting of 0.26. The Marquesas data indicate a slower breakup of schools, a large number of schools in the area, and equal propensity of marked and unmarked fish to bite. The Fiji-a data indicate an intermediate value for the school breakup rate, although with the rate of 0.45, schools would still mix in a matter of days.

Figure 1 shows the likelihood contours for the Fiji-a data set when the vulnerability is held constant at 1.0. Contours are the natural logarithm of the likelihood times -1. Since the negative log likelihood is asymptotically chi-squared distributed, the contour labeled 46 will be a rough 95% confidence bound.

There is obviously a trade-off between the number of schools and the vulnerability. Therefore, I have held the vulnerability constant at 0.3 for each data set and redone the parameter estimation. In the two cases where the three-parameter estimate produced a vulnerability near 1.0, the two-parameter case with vulnerability fixed at 0.3 produced little change in the school breakup rate, but reduced the estimated number of schools. Thus, I have considerable confidence regarding the breakup rate, and much less confidence about the vulnerability and density of schools.

The model I have used does not allow for mortality, or emigration. Given the short period covered in these data sets, mortality may not be of much importance, but emigration may very well be quite important because the areas were small. However, as in all tagging studies, there is almost total confounding between mortality of tagged fish, tag loss, tagging mortality, and reduced probability of capture of tagged fish. Thus, while I could, in theory, add mortality and immigration to the model, it would be confounded with the parameter V. Thus, V should be thought of as both emigration and mortality in addition to reduced probability of capture.

Discussion

Although the instantaneous breakup rate for schools estimated ranges from 1.0 to 0.18 (finite rates of 16–63% per day), these rates all indicate that the individuals between schools are mixing very rapidly. Schools do not appear to remain composed of the same individuals for more than a few weeks. Breakup rates are higher than indicated by either Lester et al. (1985) or Bayliff (1988). However, these studies, as well as the results presented here, suggest that schools are not long-term stable entities. It is of course possible that skipjack behave differently in one area than in another. Thus, we need not think that the school mixing rate is a constant parameter of skipjack tuna, but rather it may differ from place to place and from season to season. However, there is no evidence in the data examined to indicate that tuna schools are long-term associations of the same individuals. Rather, there is good evidence from three locations and times that mixing takes place extremely quickly.

The major uncertainty of course is what is a school. Field staff involved in this tagging project suggest that it was quite possible that several "schools" were tagged simultaneously and recorded as a single school. Similarly, as Sharp (1981) suggested, what is traditionally called a school may in fact consist of several core groups that are very cohesive. The analysis I present and indeed the technique itself cannot determine the substructure of the school. All we can really say is that there does appear to be good evidence that what were called "schools" by the Skipjack Programme did exchange individuals quite rapidly. We cannot discount the possibility that these schools consist of cohesive subunits.

This rapid mixing may have significant implications for some fishing strategies. It suggests, for instance, that a vessel that takes only a portion of the fish in a school each day might be able to continue to fish that school for several days, relying on recruitment from other schools to maintain the school size. Fishing around fish aggregation devices (FAD's), a technique now commonly used in the western Pacific, can be thought of as such a strategy (Hilborn and Medley 1989). The results presented above suggest that if we were to monitor the tuna associated with a FAD, the composition would change considerably from day to day. Therefore, it is possible that a FAD may be fished on a rather sustained basis. Experimentation with FAD's is the next obvious step to pursue this line of research.

The analysis of exchange rates begs the question of the cause of school formation. While we may be able to rule out some simple hypotheses such as schools consisting of siblings who stay together for their entire life, we cannot rule out many other hypotheses. Alternative hypotheses for school formation include the presence of individuals who are school leaders around whom schools form, school formation as an attraction phenomenon such as modeled by Anderson (1981), and schools as random feeding associations aggregating around food. Discrimination among these hypotheses would likely be very difficult.

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References

ANDERSON, J. J. 1981. A stochastic model for the size of fish schools. Fish. Bull. U.S. 79: 315–323.

BAYLIFF, W. H. 1988. Integrity of schools of skipjack tuna, *Katsuwonus pelamis*, from the eastern Pacific Ocean as determined from tagging data. Fish. Bull. U.S. 86: 631–643.

BREDER, C. M. JR. 1976. Fish schools as operational structures. Fish. Bull. U.S. 74: 471–502.

CAYRÉ, P. 1981. Que'est-ce qu'un banc de listao (*Katsuwonus pelamis*)? Quelques reflexiones a partir des observations faites lor des campagne de marquage. Int. Comm. Conserv. Atl. Tunas, Madrid, Collect. Vol. Sci. Pap. 17: 467–470.

GILLETT, R. D., AND R. E. KEARNEY. 1983. An assessment of the skipjack and baitfish resources of French Polynesia. Skipjack Survey and Assessment Programme Final Country Report No. 7, South Pacific Commission, Noumea, New Caledonia. ix + 81 p.

HILBORN, R., AND P. MEDLEY. 1989. Tuna purse seine fishing with fish aggregating devices (FAD): models of tuna FAD interactions. Can. J. Fish. Aquat. Sci. 46: 28–32.

INTER-AMERICAN TROPICAL TUNA COMMISSION (IATTC). 1983. Annual Report — 1982. Inter-American Tropical Tuna Commission. La Jolla, CA.

KEARNEY, R. E. 1982. An assessment of the skipjack and baitfish resources of Fiji. Skipjack Survey and Assessment Programme Final Country Report No. 1, South Pacific Commission, Noumea, New Caledonia. viii + 47 p.

1983. Assessment of the skipjack and baitfish resources in the central and western tropical Pacific Ocean: a summary of the Skipjack Survey and Assessment Programme. Special Publication, South Pacific Commission, Noumea, New Caledonia. 37 p.

LESTER, R. J. G., A. BARNES, AND G. HABIB. 1985. Parasites of skipjack tuna, *Katsuwonus pelamis*. fishery implications. Fish. Bull. U.S. 83: 343–356.

OKUBO, A., W. SAKAMOTO, T. INAGAKI, AND T. KUROKI, 1977. Studies on the school behavior of fish — V. Note on the dynamics of fish schooling. Bull. Jpn. Soc. Sci. Fish. 43: 1369–1377.

SEBER, G. A. F. 1982. The estimation of animal abundance. 2nd ed. Macmillan, New York, NY. 654 p.

SHARP, G. D. 1981. What is a tuna school? Introductory document of ICCAT/SCRS symposium on definition of tuna and billfish habitats and effects of environmental variation on apparent abundance and vulnerability to fisheries. Tenerife, Canary Islands, November 5, 1981. FAO sponsored.

SMITH, P. E. 1970. The horizontal dimensions and abundance of fish schools in the upper mixed layer as measured by sonar, p. 563–591. *In* G. B. Farquhar [ed.] Proceedings of the International Symposium on Biological Sound Scattering in the Ocean. Maury Cent. Ocean Sci., Dep. Davy, Washington, DC.

SQUIRE, J. L. JR. 1978. Northern anchovy school shapes as related to problems in school size estimation. Fish. Bull. U.S. 76: 443–448.

TUNA PROGRAMME. 1984. An assessment of the skipjack and baitfish resources of Wallis and Futuna. Skipjack Survey and Assessment Programme Final Country Report No. 19, South Pacific Commission, Noumea, New Caledonia.

Picoplankton Associations in an Ultra-Oligotrophic Lake on Vancouver Island, British Columbia

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Klut, M. E., and J. G. Stockner. 1991. Picoplankton associations in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. 48: 1092–1099.

Epiphytic associations or "microaggregates" of picoplankton with larger phytoplankton in Sproat Lake, British Columbia, occurred in the epilimnion throughout the 1-yr study, but were most frequently seen at times of severe nutrient limitation in summer and autumn. Both bacterial and algal picoplankton epiphytes were attached to the cell surface of algal hosts by either fine protrusions or by their fibrillar extracellular matrix (glycocalyx). This surface coat increased in thickness as ambient nutrient concentrations became limiting, and harboured a range of heterogeneous organic and inorganic particles and phytoplankters. The phytoplanktonic host cells for these picoplankton associations included filamentous cyanobacteria, dinoflagellates, chlorophytes, coccolithophores, and diatoms. Under controlled nutrient-depleted conditions, the filamentous nitrogen-fixing cyanobacterium *Anabaena flos-aquae* became a common host of chroococcoid cyanobacterium. Some picoplankters were endophytic within the cytoplasm and vacuoles of protozoa and microflagellates, serving either as prey or as symbionts. These associations are thought to facilitate rapid carbon and nutrient recycling in pelagic ecosystems.

Pendant toute la durée de l'expérience d'un an, le picoplancton de l'épilimnion du lac Sproat (Colombie-Britannique) a formé des associations épiphytiques ou "microaggrégats" avec du phytoplancton de plus grande taille. Ces associations ont le plus souvent été observées pendant les périodes de disette de bioéléments en été et en automne. Le picoplancton bactérien et algal épiphyte s'attachait à la surface cellulaire de l'hôte par de fines protrusions ou par leur matrice extracellulaire fibrillaire (le glycocalyx). L'épaisseur de cette couche superficielle a augmenté quand les concentrations ambiantes de bioéléments sont devenues limitatives. Elle abritait aussi une variété de particules organiques et inorganiques hétérogènes et diverses espèces de phytoplancton. Les cellules phytoplanctoniques hôtes du picoplancton comprenaient des cyanobactéries filamenteuses, des dinoflagellés, des chlorophytes, des coccolithophoracées et des diatomées. Dans des conditions expérimentales d'appauvrissement en azote, *Anabaena flos-aquae*, une cyanobactérie filamenteuse fixatrice d'azote, est devenue un hôte commun de la cyanobactérie chroococcale. Certaines espèces de picoplancton sont devenues endophytes à l'intérieur du cytoplasme ou des vacuoles de protozoaires et de flagellés, soit come proie ou symbiote. On croit que ces associations facilitent le recyclage rapide du carbone et de l'azote dans les écosystèmes pélagiques.

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Heterotrophic (bacteria) and phototrophic (algae) picoplankters, although very small (0.2–2.0 μm), are known to be very important contributors to the carbon metabolism of planktonic communities of oligotrophic freshwater ecosystems (Stockner and Antia 1986; Stockner and Shortreed 1989). They can occur as free-living organisms or become associated with organic and inorganic particles and other organisms (microaggregates). It has recently been shown that picoplankters can also interact with virus-like particles (Klut and Stockner 1990).

The association of bacteria and pelagic detritus with phytoplankters, often referred to as macroscopic aggregates or "marine snow," has been previously observed and regarded as a complex and ubiquitous phenomenon of marine habitats (Alldredge 1979; Silver et al. 1986). However, very little is known of algal associations in freshwater ecosystems (Paerl 1982). The present study, using light and electron microscopy, had three basic objectives: (a) to identify and characterize the microaggregates of Sproat Lake, British Columbia, (b) to study their seasonal variation, and (c) to consider some of the processes involved in their formation and maintenance as well as some possible ecological implications.

Description of Study Lake

Sproat Lake is a large ultra-oligotrophic lake located in the south-central region of Vancouver Island (49°14′N, 125°06′W), an area with warm, dry summers and cool, wet winters. The lake has a warm-monomictic circulation pattern, seldom freezes in winter, has a mean depth of 59 m, and a theoretical water residence time of 8 yr (Shortreed and Stockner 1990). Because of surrounding mountain topography there is relatively little wind mixing and the lake is strongly stratified for up to 9 mo of the year with a relatively stable epilimnion depth of 10 m. The lake has a deep chlorophyll maximum (DCM) in the hypolimnion at a depth of between 21 and 25 m from June to November. The Sproat Lake drainage basin has shallow, sandy soils underlain by impervious igneous bedrock which results in low dissolved ion concentrations and very low biological productivity (Table 1). The lake is an important nursery area for sock-

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TABLE 1. Some salient physical, chemical, biological, and morphological variables for Sproat Lake. Values are seasonal epilimnetic averages for 1985.

Mean depth (m)	59
Surface area (km^2)	41
Residence time (yr)	8
Compensation depth (m)	21.4
Total dissolved solids ($mg \cdot L^{-1}$)	35
Total phosphorus ($\mu g \cdot L^{-1}$)	1.5
Nitrate nitrogen ($\mu g \cdot L^{-1}$)	3.0
Chlorophyll ($\mu g \cdot L^{-1}$)	1.5
Primary production ($mg\ C \cdot m^{-3} \cdot h^{-1}$)	0.53
Zooplankton biomass ($mg\ dry\ wt \cdot m^{-3}$)	10.1

eye salmon (*Oncorhynchus nerka*), which spend 1–2 yr in the lake prior to their seaward migration.

Methods

Once every 2 mo, water samples were taken from Sproat Lake at a depth of 1 m (surface) and 22 m (compensation depth) from October 1987 to September 1988. Samples were kept cool until processed, usually within 24 h of collection. A few selected samples were kept for 3 mo, without nutrient addition, in an incubator at 18°C under a 12 h light/dark cycle.

Light Microscopy

Water samples were observed with Nomarski optics using a Zeiss compound microscope.

Transmission Electron Microscopy (TEM)

Cell samples concentrated on a 0.2-µm Sartorius membrane filter were prefixed for 5 min with a mixture of 2% glutaraldehyde (GA) and 1% osmium tetroxide (OsO_4) using sodium cacodylate buffer (0.1 M, pH 7.0). Specimens were then fixed for 1.5 h with GA (2.5%) containing 0.01% Alcian Blue. After washing with buffer, specimens were postfixed at room temperature for 2 h with OsO_4 (1%) containing 0.01% Ruthenium Red. Dehydrated samples were embedded in Epon 812. Ultrathin sections were viewed with a Zeiss EM 10A microscope. For negative staining, centrifuged unfixed samples were mounted on formvar-coated grids and stained with uranyl acetate (1.5%).

Scanning Electron Microscopy (SEM)

From each water sample, specimens were concentrated on three different 0.2-µm Nuclepore filters and were fixed at room temperature for 1 h with OsO_4 (1%) using sodium cacodylate (0.1 M, pH 7.0). Fixed samples were dehydrated, critical-point dried, gold coated, and then examined with a Cambridge Stereoscan 250 T electron microscope.

Results

Electron microscopy studies of Sproat Lake samples showed an abundance of picoplankton cells with an acidic polysaccharide surface coat revealed with uranyl acetate (Fig. 1, 2) or the combination of Alcian Blue with Ruthenium Red (Fig. 3, 6, 8). In some cases (e.g. *Synechococcus*) this fibrillar layer increased in thickness (approximately 2 times) when ambient nitrate levels in the lake fell from 35–40 $\mu g\ N \cdot L^{-1}$ in early spring to below detection levels in August–September. This layer can gather considerable amounts of heterogeneous particulate matter (detritus, virus-like particles, Fig. 2, 4), including a variety of microorganisms (Fig. 5–8). Water samples collected in March often contained the cyanobacterial picoplankter *Synechococcus* with anionic electron-dense deposits in the mucilaginous layer (arrowheads, Fig. 8A, 8B). Single and clustered cells were frequently observed trapped within fibrillar nets that were dispersed throughout the samples (Fig. 7).

Picoplankton epibionts were present on about 15% of the cells examined throughout much of the year but occurred on >75% of cells in September samples from both the epilimnion and DCM. They adhered to the surface of some phytoplankton cells via either fine protrusions (arrowheads, Fig. 9, 16), the glycocalyx (Fig. 13), or the peduncle of the holdfast in some bacteria (Fig. 15). The most abundant host phytoplankton cells were diatoms, notably *Rhizosolenia*, *Cyclotella*, and *Melosira*. However, chlorophytes, prymnesiophytes, dinoflagellates, and filamentous cyanobacteria also acted as hosts. Both the diatoms *Nitzschia* and *Melosira* had picoplankters attached to their surface coat (Fig. 9–12), but *Melosira* also was observed with larger nanoplankters (2.0–20.0 µm) and protozoa on the valve or girdle bands (Fig. 10–12). *Rhizosolenia* exhibited picoplankton epibionts of different sizes and shapes (Fig. 13–18), some of which were embedded in a mucilaginous material apparently released by the cells via surface pores and then adsorbed to the cell wall (arrows, Fig. 18).

The cyanobacterial epibionts of *Cyclotella* sp. showed both smooth and undulated outer surfaces (Fig. 19–23). Although abundant at 22 m in the DCM, they also occurred throughout the epilimnion. These epibionts were seen attached to the surface of the valves as well as on the intercalary bands (girdle) (Fig. 19, 20, 22–24), while some appeared to be entering the cell through the connecting band of the epitheca (arrowheads, Fig. 20, 21).

Light microscopic observations indicated that the chlorophyte *Pediastrum integrum* is embedded in an extensive mucilaginous matrix populated by large numbers of unidentified microorganisms and particles (Fig. 25). In addition, SEM studies showed that rod-shaped bacteria (presumably bacilli) were attached to the surface of coccolithophores (arrowhead, Fig. 26) or located in the sulcus groove of dinoflagellates (e.g. *Peridinium inconspicuous*, arrowheads, Fig. 27).

Studies on water samples kept for 3 mo in the incubator without adding nutrients showed that the nitrogen-fixing filamentous cyanobacterium *Anabaena flos-aquae* became dominant. Chroococcoid cyanobacteria were attached to the surface of a trichome cell near the heterocyst (usually one or two cells away from the heterocyst (H), Fig. 28, 29) that was terminal and clearly identified by its differential shape, thick wall, and hyaline appearance. Additional TEM studies showed that senescent *Botryococcus* cells were found both inside vacuoles or apparently being phagocytosed by a sarcodine protozoan (Fig. 30–32).

Among the endophytic associations observed, bacteria were found inside the cytoplasm and within food vacuoles of heterotrophic microflagellates (e.g. arrowheads, Fig. 33). They were also found within the cytoplasm of dinoflagellates (arrowheads, Fig. 34, 35).

Discussion

This seasonal study has focused on epiphytic picoplanktonic associations (microaggregates) in Sproat Lake and the role of

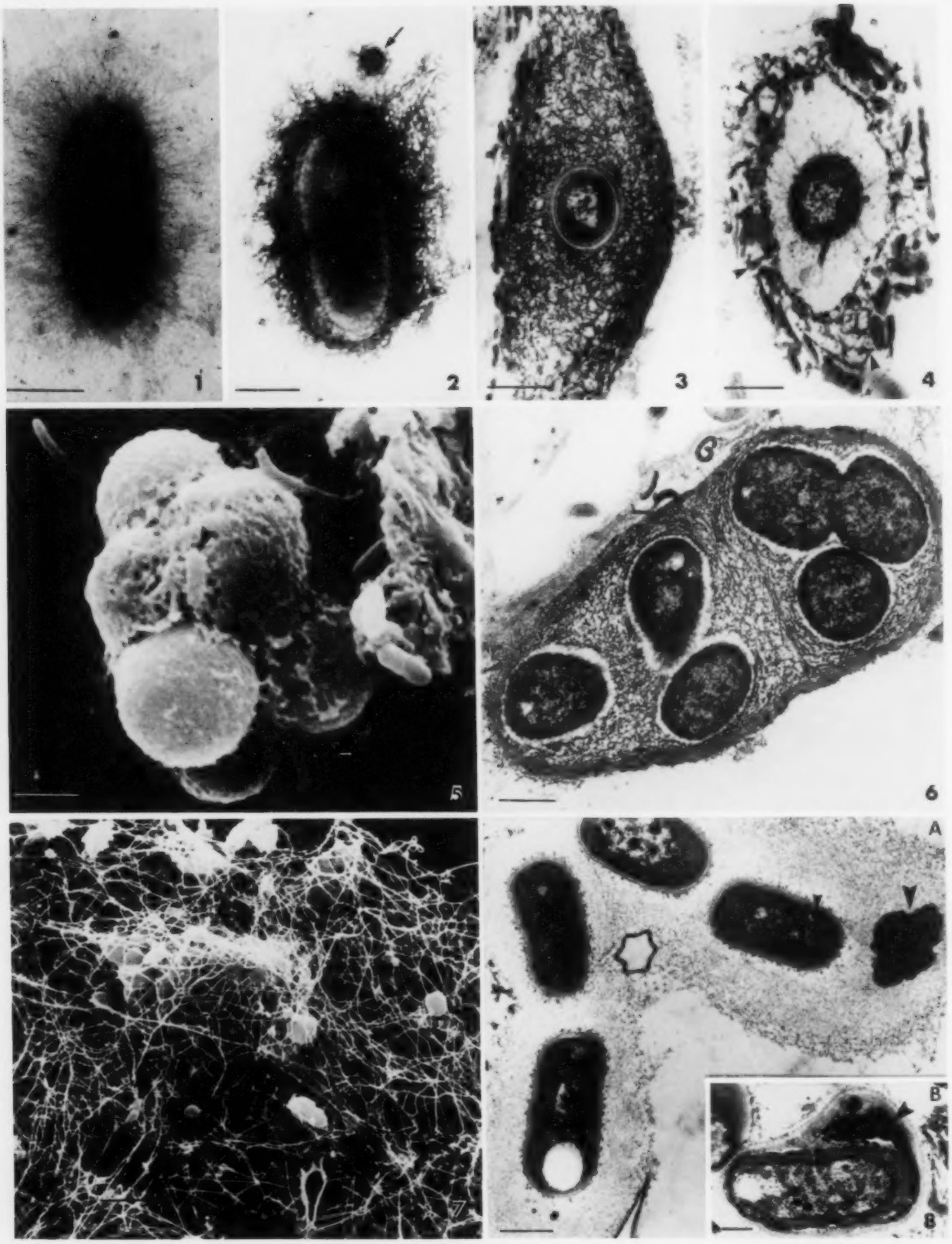


FIG. 1. and 2. Cyanobacterium negatively stained with uranyl acetate. Note a virus-like particle adhered to the cell surface coat (arrow). Bars = 0.4 and 0.3 μm, respectively. FIG. 3. Cyanobacterium surrounded by the fibrillar matrix stained with Alcian Blue – Ruthenium Red. Bar = 0.3 μm). FIG. 4. Particulate matter attached to the surface layer of a cyanobacterium. Bar = 0.3 μm. FIG. 5. SEM of microaggregate. Note the bacterium adhered to the mucilaginous coat. Bar = 1.2 μm. FIG. 6. TEM of a microaggregate embedded in the matrix stained with Alcian Blue – Ruthenium Red. Bar = 0.4 μm. FIG. 7. Some picoplankters trapped in a fibrillar net. Bar = 1.5 μm. FIG. 8A and 8B. *Synechococcus* sp. collected in April containing electron-dense material (arrowheads) inside and outside the cell. Bars = 0.4 and 0.3 μm, respectively.

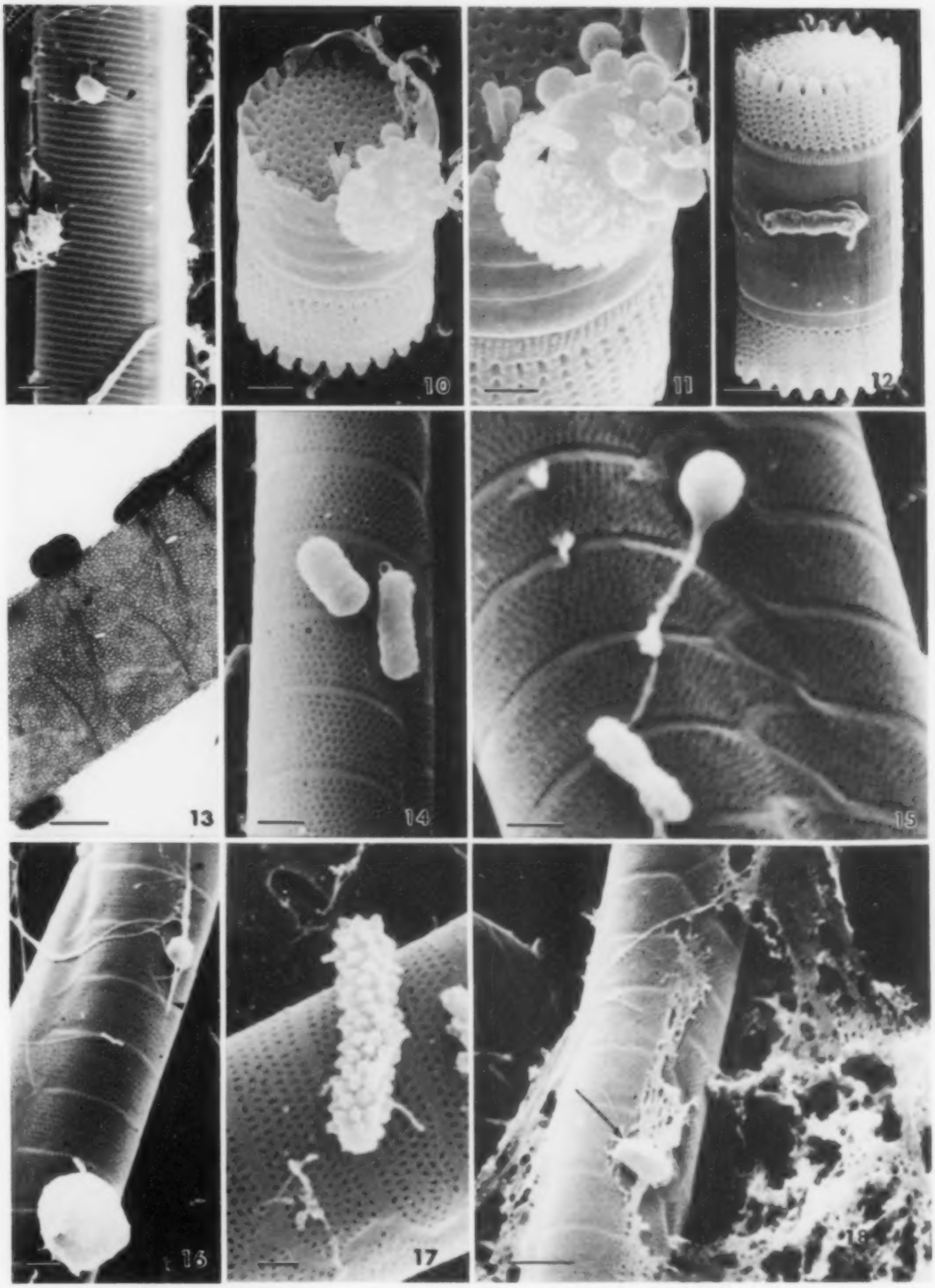
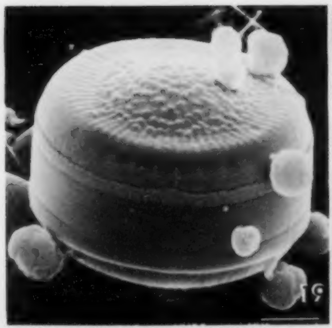
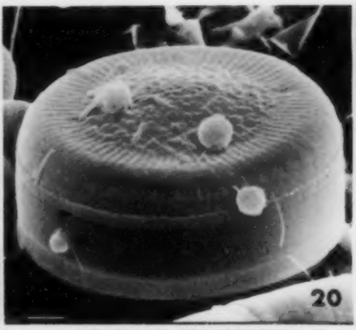
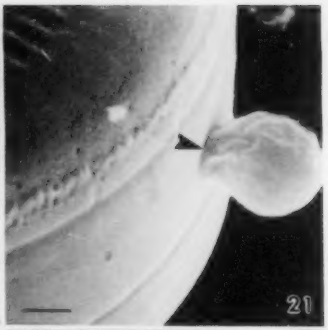
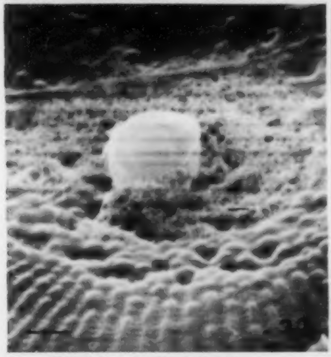


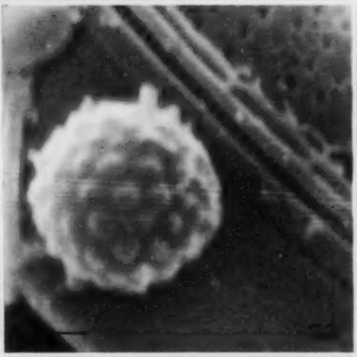
FIG. 9. Picoplankter attached via fine protrusions (arrowheads) to the surface of *Nitzschia* sp. Bar = 0.3 μm. FIG. 10 and 11. Epibionts of *Melosira* sp. in August. Bars = 2.0 and 1.0 μm, respectively. FIG. 12. Protozoan on the girdle band of *Melosira* sp. Bar = 3.0 μm. FIG. 13–18. Various picoplankters adhered to the surface of *Rhizosolenia* sp. Arrowheads on Fig. 16 denote fine protrusions. On FIG. 18, note the epibiont embedded in the extracellular matrix. Bars = 1.0, 0.5, 0.8, 1.0, 0.5, and 1.0 μm, respectively.

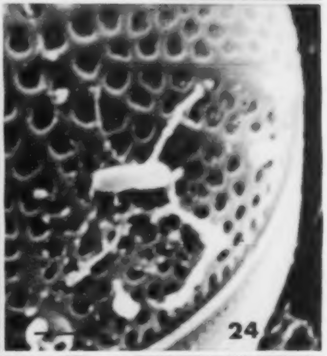


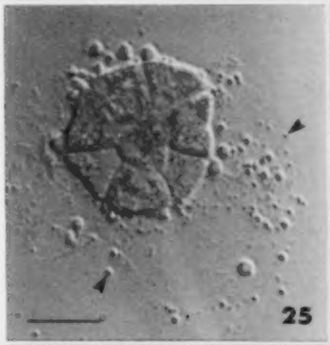


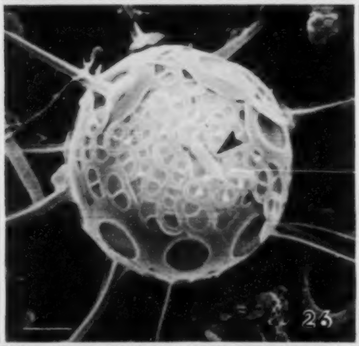


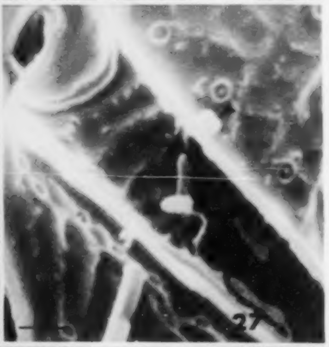


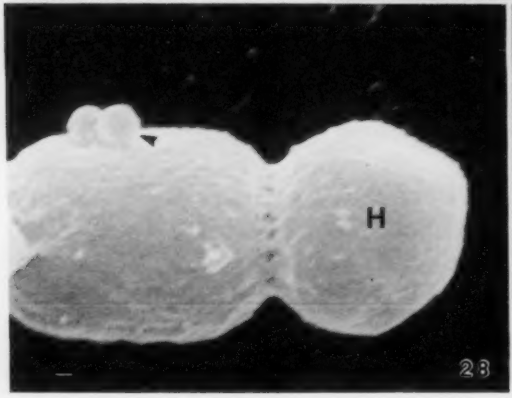












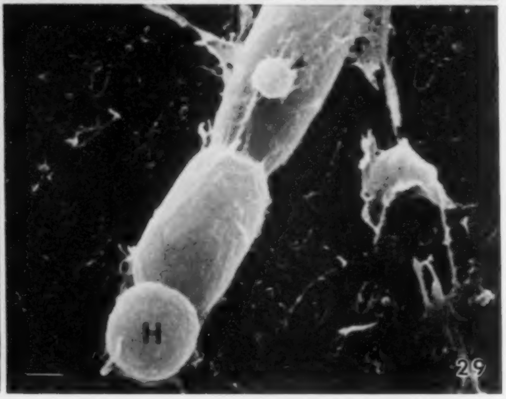
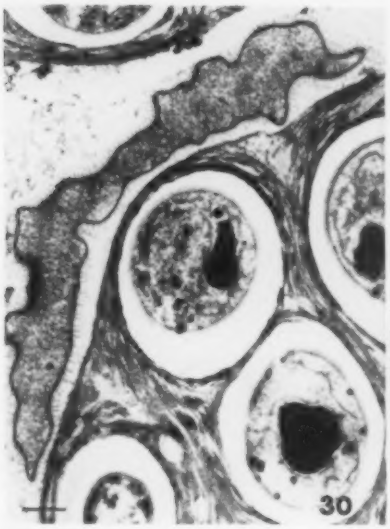


FIG. 19–24. Epibionts of *Cyclotella* sp. in September. Arrowheads on Fig. 20 and 21 denote apparent sites of phagocytosis through the intercalary bands. In Fig. 22, note the mucilagenous material around the cyanobacterium. Figure 23 shows an epiphytic chroococcoid cyanobacterium with an undulated surface. Bars = 3.0, 4.2, 4.0, 1.0, 0.4, and 0.5 μm, respectively. FIG. 25. Various microorganisms trapped in the mucilagenous matrix of *Pediastrum integrum* (arrowheads). Bar = 18 μm. FIG. 26. A picoplankton cell (arrowhead) attached to the surface of a coccolithophore. Bar = 1.5 μm. FIG. 27. Note the presence of picoplankters in the groove area of *Peridinium inconspicuous*. Bar = 1.2 μm. FIG. 28 and 29. Epiphytic chroococcoid cyanobacteria close to the heterocyst (H) of *Anabaena flos-aquae*. Bars = 0.6 and 0.5 μm, respectively.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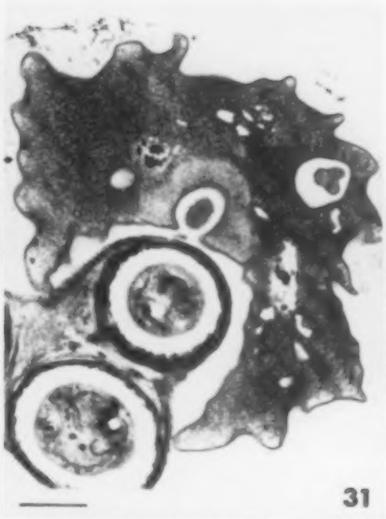
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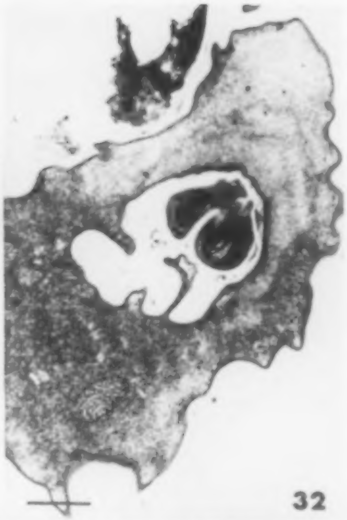
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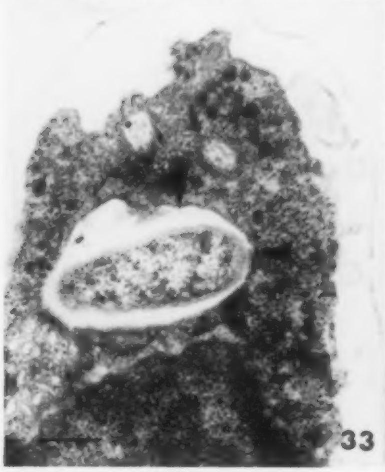
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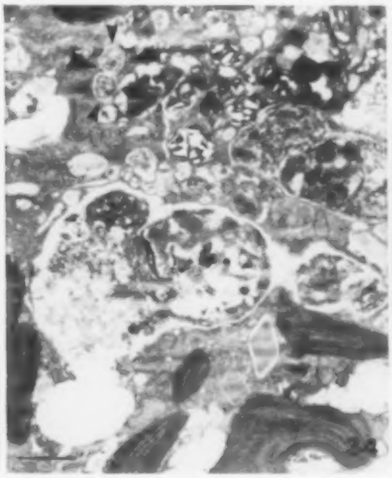
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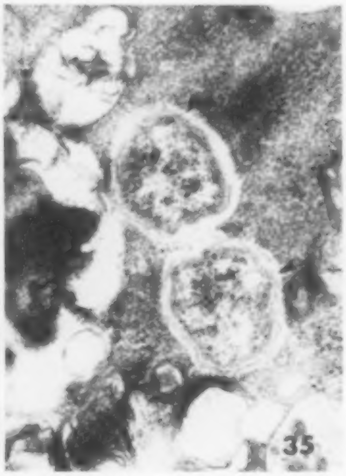
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FIG. 30–32. Three different stages of a sarcodine protozoa phagocytosing *Botryococcus* sp. cells. Bars = 0.6, 0.1, and 1.0 μm, respectively. FIG. 33. Bacterium within a vacuole of a heterotrophic microflagellate. Bar = 0.3 μm. FIG. 34 and 35. A dividing bacterium (arrowheads) inside the cytoplasm of a dinoflagellate cell. Bars = 0.5 and 0.2 μm, respectively.

the surface coat (glycocalyx) in their formation and maintenance. Our light and electron microscopic observations of concentrated water samples showed picoplankters associated with both detritus and nano- and microphytoplankton (20–200 μm).

Using both uranyl acetate and the cationic dyes Alcian Blue – Ruthenium Red, we have found that most procaryotic and eucaryotic cells in the epilimnion and DCM of Sproat Lake have an acidic, carbohydrate-rich fibrillar matrix (glycocalyx) whose thickness and morphological appearance change with fluctuating nutrient levels. It has been suggested previously that the glycocalyx plays an important role in regulating essential activities such as cellular uptake, recognition, and adhesion (Klut et al. 1985). Some have opined that the fibrillar (hairy) sheath of some algae acts as an organic carrier system for the uptake or exchange of ionic nutrients (Lange 1973; Lean 1976; Leppard et al. 1977; Leppard and Burnison 1983).

In Sproat Lake samples collected in April, the rod-shaped cyanobacterium *Synechococcus* contained some anionic, electron-dense deposits both inside the cells (polyphosphate bodies) and in localized areas of their cellular matrix (Fig. 8B). It is well known that under favourable nutrient conditions many algal species can take up and store an excess of phosphate (Cembella et al. 1985). While a major portion of this ion can be stored inside the cells as polyphosphate, e.g. in some cyanophytes, chlorophytes, and diatoms (Lean and Nalewajko 1976; Cembella et al. 1985; Klut et al. 1989), minor, although significant, quantities can be excreted, presumably in the form of dissolved organic or colloidal phosphorus, and deposited in the cell surface matrix (Lean and Nalewajko 1976; Pearl and Lean 1976). Whether the observed electron-opaque material indicates the presence of precipitated phosphate, other inorganic ions, or anionic exudates is at present unclear.

Many have noted that the increase in thickness of the extracellular matrix of some algae is a specific morphogenetic response to nutrient shortages (Sinclair and Whitton 1977; Whitton and Harding 1978; Klut et al. 1985; Gibson and Whitton 1987). It is expected that by increasing the area to volume ratio, single cells improve their efficiency for nutrient absorption as well as their buoyancy. Improved buoyancy may counteract sinking and increase the likelihood of encounters with detritus and other microorganisms which may trigger the formation of microaggregates as those observed in Sproat Lake. Currently, there is very little known about these microaggregates because of the fragile nature of their embedding matrix and the difficulty in its preservation and recognition using filtration pressure and standard electron microscopy procedures (Lean 1976; Burnison and Leppard 1983). Although some cell aggregates from Sproat Lake seemed to occur throughout the year, they were definitely more abundant in late summer when NO_3 levels were below detection limits (dissolved PO_4 levels remain at undetectable levels throughout the year (Shortreed and Stockner 1990)).

It is known that a decline in the concentration of external available nutrients may also lead to an increase in the production of "cell exudates" (nondiffusible secretory substances released by the cells and subsequently adsorbed to the cell surface). Among other functions, cell exudates can (a) alter cell adherence properties (Wrangstadh et al. 1986), (b) act as carrier molecules for iron transport, e.g. siderophores (Murphy et al. 1976; Simpson and Neilands 1976), (c) serve as metabolic regulators, such as cyclic 3′,5′-AMP (Azam and Ammerman 1984), and (d) create "microzones" rich in organic matter (Fogg 1966; Huntsman 1972; Paerl 1985). These organic nutrient-enriched surface areas can act as rich metabolite pools, attracting and enhancing the growth of bacteria and microflagellates (Bell and Lang 1974; Biddanda and Pomeroy 1988; Sieburth 1988). We believe that in Sproat Lake with the onset of nutrient limitation in early summer (June–July), microaggregates become more abundant in the epilimnion because they provide a self-sustaining microcosm that offers a competitive advantage over the free-living condition (Goldman 1984; Sieburth 1988). In addition to field observations of microaggregate formation, our laboratory incubator results showed that during periods of nutrient exhaustion, surviving chroococcoid cyanobacteria were closely associated with the N_2-fixing cyanobacterium *Anabaena flos-aquae*. Whether the epiphytic coccoid cyanobacterium is using fixed nitrogen or stimulating its fixation is not known.

In Sproat Lake, both cyanobacterial and bacterial picoplankters were found as epiphytes on both actively growing (healthy) and senescent phytoplankters. As primary decomposers of particulate and dissolved organic matter, bacteria–phytoplankton associations (e.g. detritus) are not surprising, but it is well known that bacteria can also be of mutual benefit to healthy algal hosts by (a) supplying them with metabolic effectors (e.g. limiting CO_2, vitamins, amino acids, growth factors, etc.) (Taylor 1982; Hall and Fisher 1983; Azam and Ammerman 1984; Paerl 1985; Newhook and Briand 1987), (b) immobilizing exudates (e.g. allelopathic substances) and indirectly promoting algal growth (Newhook and Briand 1987) (c) recycling nutrients (Azam and Ammerman 1984; Newhook and Briand 1987), (d) serving as a major or alternative source of carbon for heterotrophic and mixotrophic microflagellates (Doddema and Veer 1983; Porter et al. 1985; Bird and Kalff 1986; Biddanda 1988), and (e) "propelling" them (e.g. diatoms) (Taylor 1982). A general literature survey has shown that the epiphytic type of planktonic associations are mainly reported from marine waters (Bell and Lang 1974; Taylor 1982; Biddanda 1986; Silver et al. 1986; Biddanda and Pomeroy 1988) and only rarely from freshwater (Sheath and Morrison 1982).

We also observed endophytic associations, where picoplankton were either prey for heterotrophic microflagellates and found inside food vacuoles or were symbiotically associated with larger phytoplankton cells. Endophytic bacteria have previously been found in the periplasmic space of cyanobacteria (Wujek 1979; Wilkinson 1979), in localized areas of the cytoplasm of chloromonads (Heywood 1978), within the nucleus, chloroplast, or cytoplasm of dinoflagellates (Silva and Franca 1985; Chesnick and Cox 1986; Wilcox 1986), inside the nucleus of euglenoids (see Taylor 1982), and within the endoplasmic reticulum of chrysophytes (Doddema and Veer 1983). In addition, a filamentous cyanobacterium (*Richelia intracellularis*) has been reported endosymbiotically associated with *Rhizosolenia* (Weare et al. 1974; Sundstrom 1984).

Very little is known about the physiological or adaptive significance of the various picoplankter–phytoplankton associations described here. However, earlier observations (Klut and Stockner 1990) and the present study have shown that pelagic associations (microaggregates) are common in Sproat Lake and that they have a seasonal component that appears to be related to the sharp late June decline in nitrate in the epilimnion. We think that their occurrence is not the consequence of simple, random physical contact and adherence between dying or senescent cells, but rather represents a mode of existence with high survival value, as it represents a potentially important metabolic alternative to maintain cell growth in the face of severe nutrient depletion. Free-living picoplankters by virtue of their small size avoid grazing by many macrozooplankton (Stockner and Porter 1988). Larger picoplankton–phytoplankton associations are likely to be more susceptible to grazing, thereby enhancing the carbon flow from picoplankton to metazoans in pelagic food webs of ultra-oligotrophic systems (Pomeroy 1984; Sieburth 1988).

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References

ALLDREDGE, A. L. 1979. The chemical composition of macroscopic aggregates in two neretic seas. Limnol. Oceanogr. 24: 855–866.

AZAM, F., AND J. W. AMMERMAN. 1984. Cycling of organic matter by bacterioplankton in pelagic marine ecosystems: microenvironmental considerations, p. 345–360. *In* M. R. Fasham [ed.] Flow of energy and materials in marine ecosystems — theory and practice. Plenum Press, London. 733 p.

BELL, W. H., AND J. M. LANG. 1974. Selective stimulation of marine bacteria by algal extracellular products. Limnol. Oceanogr. 19: 933–939.

BIDDANDA, B. A. 1986. Structure and function of marine microbial aggregates. Oceanol. Acta 9(2): 209–211.

1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. II. Microbial metabolism. Mar. Ecol. Prog. Ser. 42: 89–95.

BIDDANDA, B. A., AND L. R. POMEROY. 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. I. Microbial succession. Mar. Ecol. Prog. Ser. 42: 79–88.

BIRD, D. F., AND J. KALFF. 1986. Bacterial grazing by planktonic algae. Science (Wash., DC) 231: 493–495.

BURNISON, B. K., AND G. G. LEPPARD. 1983. Isolation of colloidal fibrils from lake water by physical separation techniques. Can. J. Fish. Aquat. Sci. 40: 373–381.

CEMBELLA, A. D., N. J. ANTIA, AND P. J. HARRISON. 1985. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective — Part 2. CRC Crit. Rev. Microbiol. 11: 13–81.

Chesnick, J. M., and E. R. Cox. 1986. Specialization of endoplasmic reticulum architecture in response to a bacterial symbiosis in *Peridinium balticum* (Pyrrhophyta). J. Phycol. 22: 291–298.

Doddema, H., and J. van der Veer. 1983. *Ochromonas monicis* sp. nov., a particle feeder with bacterial endosymbionts. Cryptogam. Algol. 6: 89–97.

Fogg, G. E. 1966. The extracellular products of algae. Oceanogr. Mar. Biol. Annu. Rev. 4: 195–212.

Gibson, M. T., and M. A. Whitton. 1987. Influence of phosphorus on morphology and physiology of freshwater *Chaetophora*, *Draparnaldia* and *Stigeoclonium* (Chaetophorales, Chlorophyta). Phycologia 26: 59–69.

Goldman, J. C. 1984. Oceanic nutrient cycles, p. 137–170. *In* M. R. Fasham [ed.] Flows of energy and material in marine ecosystems — theory and practice. Plenum Press, New York, NY.

Hall, S. L., and F. M. Fisher, Jr. 1983. Organic compounds released from a natural population of epibenthic bluegreen algae. J. Phycol. 19: 365–368.

Heywood, P. 1978. Intracellular bacteria in *Gonyostomum semen* (Chloromonadophyceae). J. Phycol. 14: 121–122.

Huntsman, S. A. 1972. Organic excretion by *Dunaliella tertiolecta*. J. Phycol. 8: 59–63.

Klut, M. E., T. Bisalputra, and N. J. Antia. 1985. Some cytochemical studies on the cell surface of *Amphidinium carterae* (Dinophyceae). Protoplasma 129: 93–99.

Klut, M. E., and J. G. Stockner. 1990. Virus-like particles in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. 47: 725–730.

Klut, M. E., Stockner, J. G., and T. Bisalputra. 1989. Further use of fluorochromes in the cytochemical characterization of phytoplankton. Histochem. J. 21: 645–650.

Lange, W. 1973. Bacteria-assimilable organic compounds, phosphate, and enhanced growth of bacteria-associated blue-green algae. J. Phycol. 9: 507–509.

Lean, D. R. S. 1976. Phosphorus kinetics in lake water: influence of membrane filter pore size and low-pressure filtration. J. Fish. Res. Board Can. 33: 2800–2804.

Lean, D. R. S., and C. Nalewajko. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. J. Fish. Res. Board Can. 33: 1312–1323.

Leppard, G. G., and B. K. Burnison. 1983. Bioavailability, trace element associations with colloids and an emerging interest in colloidal organic fibrils, p. 105–122. *In* G.G. Leppard [ed.] Trace element speciation in surface waters and its ecological implications. Plenum Press, New York, NY. 320 p.

Leppard, G. G., A. Massalski, and D. R. S. Lean. 1977. Electron-opaque microscopic fibrils in lakes: their demonstration, their biological derivation and their potential significance in the redistribution of cations. Protoplasma 92: 289–309.

Murphy, T. P., D. R. S. Lean, and C. Nalewajko. 1976. Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae. Science (Wash., DC) 192: 900–902.

Newhook, R., and F. Briand. 1987. Bacteria as structuring agents in lakes: field manipulations with bacterioplankton. Arch. Hydrobiol. 109: 121–138.

Paerl, H. W. 1982. Interactions with bacteria, p. 441–461. *In* N. G. Carr and B. A. Whitton [ed.] The biology of cyanobacteria. Botanical monographs. Blackwell, London. 556 p.

1985. Microzone formation: its role in the enhancement of aquatic N_2 fixation. Limnol. Oceanogr. 30: 1246–1252.

Paerl, H. W., and D. R. S. Lean. 1976. Visual observations of phosphorus movement between algae, bacteria, and biotic particles in lake water. J. Fish. Res. Board Can. 33: 2805–2813.

Pomeroy, L. R. 1984. Significance of microorganisms in carbon and energy flow in marine ecosystems, p. 405–411. *In* M. J. Klug and C. A. Reddy [ed.] Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC. 710 p.

Porter, K. G., E. B. Sherr, B. F. Sherr, M. Pace, and R. W. Sanders. 1985. Protozoa in planktonic food webs. J. Protozool. 32: 409–415.

Sheath, R. G., and M. O. Morrison. 1982. Epiphytes on *Cladophora glomerata* in the Great Lakes and St. Lawrence seaway with particular reference to the red alga *Chroodactylon ramosum* (= *Aserocytis smargdina*). J. Phycol. 18: 385–391.

Shortreed, K. S., and J. G. Stockner. 1990. Effect of nutrient additions on distribution and density of pico-, nano-, and microphytoplankton in an oligotrophic lake with a seasonal hypolimnetic chlorophyll maximum. Can. J. Fish. Aquat. Sci. 47: 262–273.

Sieburth, J. McN. 1988. The nanoalgal peak in the dim oceanic pycnocline: is photosynthesis augmented by microparticulates and their bacterial consortia?, p. 101–130. *In* C. R. Agegian [ed.] Biogeochemical cycling and fluxes between the deep euphotic zone and other oceanic realms. NOAA Natl. Undersea Res. Prog. Res. Rep. 88-1.

Silva, S. E., and S. Franca. 1985. The association dinoflagellate–bacteria: their ultrastructural relationship in two species of dinoflagellates. Protistologica 11: 429–446.

Silver, M. A., M. M. Gowing, and P. J. Davoll. 1986. The association of photosynthetic picoplankton and ultraplankton with pelagic detritus through the water column (0–2000 m), p. 311–341. *In* T. Platt and W. K. W. Li [ed.] Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. 214: 583 p.

Simpson, F. B., and J. B. Neilands. 1976. Siderochromes in cyanophyceae: isolation and characterization of schizokinen from *Anabaena* sp. J. Phycol. 12: 44–48.

Sinclair, C., and B. A. Whitton. 1977. Influence of nutrient deficiency on hair formation in the Rivulariaceae. Br. Phycol. J. 12: 297–313.

Stockner, J. G., and N. J. Antia. 1986. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2472–2503.

Stockner, J. G., and K. G. Porter. 1988. Microbial food webs in freshwater planktonic ecoystems, p. 69–83. *In* S. R. Carpenter and J. Kitchell [ed.] Complex interactions in lake communities. Springer-Verlag, New York, NY. 383 p.

Stockner, J. G., and K. S. Shortreed. 1989. Algal picoplankton production and contribution to food-webs in oligotrophic British Columbia lakes. Hydrobiologia 173: 151–166.

Sundstrom, B. G. 1984. Observations on *Rhizosolenia clevei* Ostenfeld (Bacillariophyceae) and *Richelia intracellularis* Schmidt (Cyanophyceae). Bot. Mar. 27: 345–355.

Taylor, F. J. R. 1982. Symbioses in marine microplankton. Ann. Inst. Oceanogr. Paris 58: 61–90.

Weare, N. W., F. Azam, T. H. Mague, and O. Holm-Hansen. 1974. Microautoradiographic studies of the marine phycobionts *Rhizosolenia* and *Richelia*. J. Phycol. 10: 369–371.

Whitton, B. A., and J. P. C. Harding. 1978. Influence of nutrient deficiency on hair formation in *Stigeoclonium*. Br. Phycol. J. 13: 65–68.

Wilcox, L. W. 1986. Prokaryotic endosymbionts in the chloroplast stroma of the dinoflagellate *Woloszynskia pascheri*. Protoplasma 135: 71–79.

Wilkinson, C. R. 1979. Bdellovibrio-like parasite of cyanobacteria symbiotic in marine sponges. Arch. Microbiol. 123: 101–103.

Wrangstadh, M., P. L. Conway, and S. Kjelleberg. 1986. The production and release of an extracellular polysaccharide during starvation of a marine *Pseudomonas* sp. and the effect thereof on adhesion. Arch. Microbiol. 145: 220–227.

Wujek, D. E. 1979. Intracellular bacteria in the blue-green alga *Pleurocapsa minor*. Trans Am. Microsc. Soc. 98: 143–145.

Bias Associated with Using the Eggers Model for Estimating Fish Daily Ration

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Hayward, R. S. 1991. Bias associated with using the Eggers model for estimating fish daily ration. Can. J. Fish. Aquat. Sci. 48: 1100–1103.

Estimates of yellow perch (*Perca flavescens*) daily ration derived from Eggers' model were compared with those based on the Elliott–Persson model and a 3-h within-day sampling interval. Correcting for the bias in Eggers estimates associated with food weight differences at the start and finish of estimation periods significantly reduced discrepancies between the two when Eggers estimates were also based on a 3-h sampling interval. As sampling interval was increased to 6 and 12 h for Eggers estimates, a positive bias related to variability about $\bar{F}$ emerged and inflated exponentially with increasing ration levels. A third bias (typically positive) may be introduced when $\bar{F}$ in Eggers' model is computed without regard to the skewness that often exits in distribution of food weights among fishes within a single collection. Use of the median or a normalizing transformation will avert this. While each bias alone can have an important effect on daily ration estimates, the possibility of accentuated bias from multiple sources also exists. Two of the potential biases are easily managed, but caution is advised when Eggers' approach is used to estimate daily rations based on expanded sampling intervals.

On a comparé des estimations de la ration quotidienne de la perchaude (*Perca flavescens*) obtenues du modèle d'Eggers à celles basées sur le modèle d'Elliott–Persson (intervalle d'échantillonnage quotidien de 3 h). Après que les estimations d'Eggers ont été corrigées du biais lié aux différences de poids des aliments au début et à la fin de l'expérience, on a obtenu des écarts nettement moindres entre les deux estimations. Lorsque l'intervalle d'échantillonnage est passé à 6 à 12 h dans le cas des estimations d'Eggers, on a observé un biais positif lié à la variabilité de $\bar{F}$. Ce biais a augmenté de façon exponentielle en fonction d'une augmentation de la ration. Un troisième biais généralement positif peut survenir lorsque $\bar{F}$ dans le modèle d'Eggers est calculé sans tenir compte de l'asymétrie qui existe souvent dans la distribution des poids de nourriture parmi les poissons au cours d'un seul échantillonnage. L'utilisation de la médiane ou d'une normalisation éliminera cette possibilité. Bien que chaque biasis puisse avoir une incidence importante sur les estimations de la ration quotidienne, il existe aussi la possibilité d'un biais accentué venant de sources multiples. Deux de ces biais potentiels sont facilement éliminés, mais on recommande une certaine prudence lorsqu'on utilise la méthode d'Eggers pour déterminer la ration quotidienne basée sur un intervalle étendu d'échantillonnage.

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The value of information on food consumption rates (daily ration) for fishes has long been recognized by workers in fisheries and aquatic ecology (e.g. Bajkov 1935). Such information has been used to compare suitability of feeding environments for selected fish species (Hayward and Margraf 1987; Angradi and Griffith 1990), to estimate predation pressure placed upon prey communities by fish populations (Ruggerone 1989), and to evaluate relationships between food consumption and growth in field settings (Boisclair and Leggett 1989).

When direct estimation of fish daily ration is undertaken for a species and age group whose stomach (or gut) evacuation follows an exponential trajectory, the model of Elliott and Persson (1978) is most often used. To estimate ration on a single day within a limited area, collections of fish are required at short time intervals (typically 3 h) over 24-h periods (nine collections). The labor-intensive nature of this procedure often precludes determinations of fish daily ration, where such information is warranted, and has placed high value on developing lower-cost approaches (Hewett and Johnson 1987; Hayward and Margraf 1988).

In contrast with the Elliott–Persson model, the model of Eggers (1977) has been infrequently used in studies involving field estimation of fish daily ration despite its simple form which has advantages for variance estimation (see Boisclair and Leggett 1988). The reason for this infrequent use appears to be because its estimates are known to be biased (Eggers 1979) whenever food amounts in fish stomachs are not identical at the beginning and end of estimation periods (hereafter referred to as lack of closure). Otherwise, this model is mathematically equivalent to the Elliott–Persson model. Recently, Boisclair and Leggett (1988) suggested that lack of closure over 24-h periods may not lead to serious bias in Eggers estimates of fish daily ration inasmuch as Eggers estimates of yellow perch (*Perca flavescens*) rations (based on whole gut contents) were found not to differ significantly from comparable estimates generated by the widely accepted model of Elliott and Persson (1978). Moreover, Boisclair and Leggett (1988) provided evidence that the Eggers approach is relatively robust with respect to sampling interval (fewer fish collections required within 24 h) in comparison with the Elliott–Persson approach.

Because of the robustness indicated for the Eggers approach with respect to within-day sampling interval, I expect that the

Eggers model will be used more often in conjunction with sampling intervals exceeding 3 h in future field studies. In this paper I use diel feeding data for yellow perch to explore the behavior of Eggers daily ration estimates relative to those produced by the widely accepted Elliott–Persson approach with collections made at approximately 3-h intervals over approximately 24-h periods. In particular I examine the magnitude of lack-of-closure bias for the Eggers model (Eggers 1979), the effect of correcting for this bias, as well as the behavior of bias-corrected Eggers estimates as sampling interval is increased beyond 3 h. My findings indicate that lack-of-closure bias can and should be corrected for, but that caution is warranted when within-day sampling intervals exceed 3 h, particularly when fish are feeding at middle to high daily ration levels.

Methods

Trawl collections of yellow perch were made throughout 21- or 24-h periods at approximately 3-h intervals on eight dates (spring–fall) from 1983 through 1984 in Lake Erie at two sampling stations (Hayward and Margraf 1987, 1988). Diel collections on six of these dates yielded sufficient numbers ($N >> 5$ in most trawl collections) of both small (90–139 mm total length (TL)) and large (140–200 mm TL) yellow perch to establish two separate diel collection dates, only small- or large-sized yellow perch were retained, giving a total of 14 size-specific diel collection series. Median food weights (dry food weight per dry predator weight) were determined for small or large yellow perch within each of the eight or nine trawl collections comprising each of the 14 diel collection series. Separation into size groups was considered appropriate because size-related, 24-h food weight patterns (and consequently daily rations) were substantially different for fish collected within the same trawl hauls (Hayward and Margraf 1987, 1988).

Two estimates of yellow perch daily ration were made for each of the 14 diel collection series. One was determined from the Eggers (1977) model

$$(1)\quad \hat{C}_T = \hat{\hat{F}} \times \hat{R} \times (t_f - t_0)$$

where $\hat{C}_T$ is the estimated quantity of food consumed during period T, which begins at the time t_0 and ends at t_f, $\hat{\hat{F}}$ is the estimated mean food weight in stomachs over the estimation period, and $\hat{R}$ is the estimated rate of stomach evacuation throughout that period. The other estimate was determined from the Elliott and Persson (1978) model

$$(2)\quad \hat{C}_T = (\hat{F}_f - \hat{F}_0 e^{-\hat{R}T})\hat{R}T/(1 - e^{-\hat{R}T})$$

where $\hat{F}_0$ and $\hat{F}_f$ are estimated food weights in stomachs at the beginning (t_0) and end (t_f) of the estimation period T, respectively, and all other parameters are as previously defined. For the Eggers estimates, the estimation period (T) was the entire 21 or 24 h associated with a diel collection series, and median food weights from each of the eight or nine component trawl collections were averaged to determine $\hat{\hat{F}}$. For the Elliott–Persson estimates, median food weights from the beginning and end of each 3-h period were entered into the model to compute short-interval consumption. Total consumption over a given diel collection period was then determined by summing over all seven or eight component intervals. The same instantaneous stomach evacuation rate ($\hat{R}$) was used in both models to estimate consumption from any given diel collection series. The temperature-dependent equation of Persson (1979) was used to estimate R for yellow perch from each diel series.

Eggers (1979) showed that

$$(3)\quad \hat{F} \times R \times (t_f - t_0) = C_T - (F_f - F_0)$$

so that when $F_0 > F_f$, $\hat{C}_T$ should be positively biased, and conversely when $F_0 < F_f$. From my data set, I determined differences between median food weights at the beginning and end of each diel series ($\hat{F}_0 - \hat{F}_f$) and regressed these against differences between associated Eggers and Elliott–Persson estimates of daily ration to determine what portion of the overall discrepancies in estimates given by the two models would be attributed to the lack of closure in diel food weight patterns. This same procedure was repeated with Eggers estimates being based on diel collections that were made at approximately 6- and 12-h intervals.

To correct for lack-of-closure bias, differences between food weights at the start and finish of each diel series were subtracted from Eggers estimates of yellow perch daily ration. Bias-corrected Eggers estimates were then regressed against Elliott–Persson estimates of daily ration to explore their relative behaviors. As before, this procedure was repeated when bias-corrected Eggers estimates were based on diel collections that were made at approximately 6- and 12-h intervals.

Results

Eggers estimates of yellow perch daily ration differed from corresponding Elliott–Persson estimates by as much as 0.015 $g \cdot g^{-1} \cdot d^{-1}$ when the former were based on a 3-h within-day sampling interval and by as much as 0.025 and 0.033 $g \cdot g^{-1} \cdot d^{-1}$ (3.3% of dry body weight) when Eggers estimates were based on sampling intervals of 6 and 12 h, respectively. The differences between Eggers and Elliott–Persson estimates of C_T and differences between estimated starting and ending food weights in diel series were positively correlated for all within-day intervals used with Eggers estimates (Fig. 1). In each case, regression slopes did not differ significantly from 1 and intercepts did not differ from zero ($p = 0.12$, 0.19, and 0.32 for regressions involving 3-, 6-, and 12-h sampling intervals for Eggers estimates, respectively). These results indicated that significant portions of the discrepancies between Eggers and Elliott–Persson estimates of daily ration were attributable to the lack-of-closure bias associated with the Eggers model. Regression slopes close to 1 and y-intercepts near zero would be expected because the known bias is exactly equal to the lack of closure in diel food weights (Eggers 1979). The proportion of the total discrepancy between Eggers and Elliott–Persson daily ration estimates which was attributable to lack of closure, however, decline from 80 to 42 to 39% when Eggers estimates were based on collections of increasing sampling interval (3, 6, and 12 h, respectively).

Correction for lack-of-closure bias in Eggers estimates (subtracting ($\hat{F}_0 - \hat{F}_f$) from $\hat{C}_T$ for all Eggers estimates) reduced variances of discrepancy distributions between Eggers and Elliott–Persson estimates of daily ration in all cases; however, this reduction was only significant where the sampling interval was 3 h (F' statistic, t-test procedure (SAS Institute, Inc. 1985); $p = 0.02$, 0.42, and 0.53, for the 3-, 6-, and 12-h sampling interval cases, respectively). Significant curvilinear relationships remained between bias-corrected Eggers and Elliott–Persson estimate differences and daily ration level (Fig. 2). Bias-corrected Eggers estimates were similar to Elliott–Persson estimates at low ration levels (y-intercepts not different from zero;

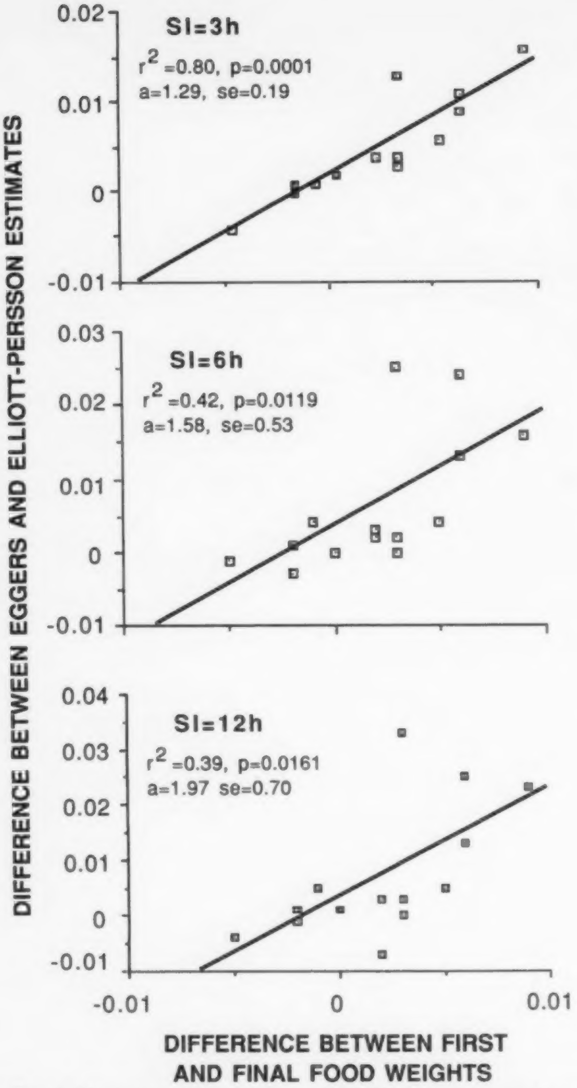

FIG. 1. Linear regressions ($y = ax + b$) of differences between Eggers and Elliott–Persson estimates of yellow perch daily ration ($g \cdot g^{-1} \cdot d^{-1}$) versus differences in median food weights in fish stomachs ($g \cdot g^{-1}$) at the start and finish of diel estimation periods. Sampling interval (SI) applies to Eggers estimates only.

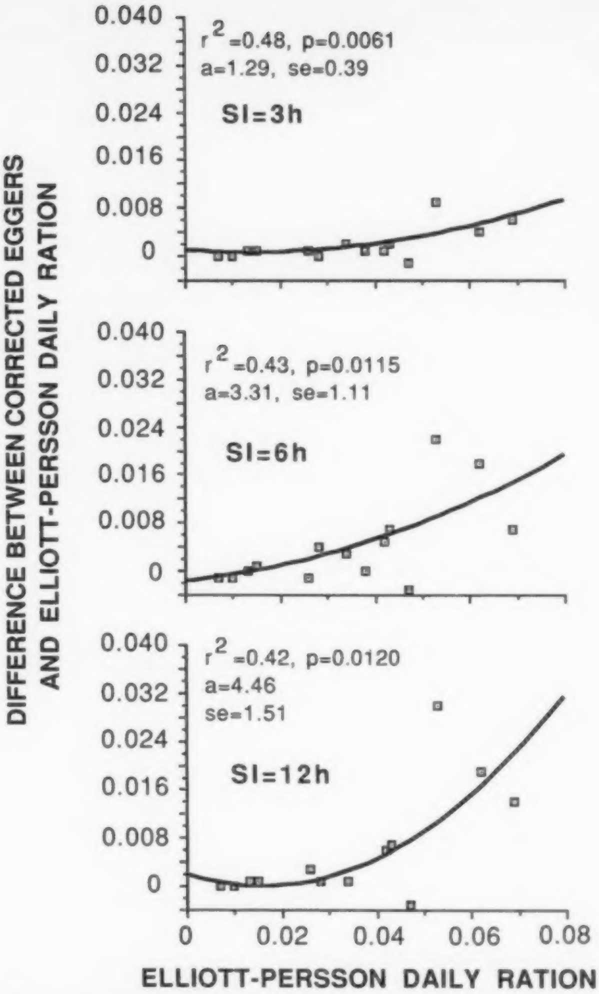

FIG. 2. Curvilinear regressions of the form $y = ax^2 + b$ where y represents differences between lack-of-closure-corrected Eggers and Elliott-Persson estimates of yellow perch daily ration ($g \cdot g^{-1} \cdot d^{-1}$) and x represents yellow perch daily ration level according to the Elliott–Persson procedure (same units). Sampling interval (ST) applies to Eggers estimates only.

$p = 0.92$, 0.73, and 0.56, for 3-, 6-, and 12-h sampling intervals, respectively) but became significantly greater as ration levels increased. Discrepancies also became more pronounced as bias-corrected Eggers estimates were based on within-day sampling intervals that increased from 3 to 12 h (analysis of covariance testing heterogeneity of slopes; $p = 0.05$). Bias-corrected Eggers estimates exceeded Elliott–Persson estimates of yellow perch daily ration by as much as 0.020 $g \cdot g^{-1} \cdot d^{-1}$ when ration levels (Elliott–Persson) were highest (near 0.070 $g \cdot g^{-1} \cdot d^{-1}$) and the sampling interval was 12 h.

Discussion

Because of the need for labor-reducing approaches for estimating fish daily ration, the robustness of the Eggers model with respect to the within-day sampling interval (fewer collections required) as purported by Boisclair and Leggett (1988) will undoubtedly lead to its more frequent use. My analysis suggests that at least two bias sources are associated with the use of the Eggers model, each of which warrants consideration whenever it is employed.

One is that due to (and exactly equal to) differences in food weights in fishes at the start and finish of the estimation period, as shown by Eggers (1979). This bias was as high as approximately 1/10 of the maximum range of daily rations for yellow perch (Kitchell et al. 1977) and accounted for a significant portion of the discrepancy between Eggers and Elliott–Persson estimates when fish collections were made every 3 h. Its relative importance as a bias source declined, however, as the sampling interval for Eggers estimates increased. This bias is easily corrected for by subtracting the difference between the starting and final food weights for the estimation period from the Eggers estimate of consumption. Surprisingly, this simple corrective measure has not been suggested in the literature.

Additional bias was indicated when Eggers daily ration estimates, after being corrected for closure bias, exceeded corresponding Elliott–Persson estimates by amounts which increased exponentially with higher ration levels. A stepwise regression analysis used to identify factors contributing to this bias source indicated that increasing variation about $\bar{F}$, which accompanied higher ration levels, was most closely related to this bias. While this bias was judged to be relatively unimportant (exceeding Elliott–Persson estimates by only 0.004 $g \cdot g^{-1} \cdot d^{-1}$ even at the highest ration level) when the sampling interval was 3 h, Eggers estimates exceeded Elliott–Persson estimates by 0.010 $g \cdot g^{-1} \cdot d^{-1}$ and more when the sampling interval was 6 h or greater and ration levels (Elliott–Persson) exceeded 0.050 $g \cdot g^{-1} \cdot d^{-1}$. This observation suggests that the robustness of the Eggers approach with respect to sampling interval may be less than was purported by Boisclair and Leggett (1988). The extent to which this bias was reduced by their working with whole gut rather than stomach contents is unknown.

It should be clarified that my reference to this systematic discrepancy between Eggers and Elliott–Persson estimates as bias associated with the Eggers approach is based upon the broad acceptance of Elliott–Persson estimates (when fish whose stomach or gut evacuation patterns are well described by an exponential decay function are collected at 3-h intervals) as standards against which other estimates may be compared (Cochran 1979; Boisclair and Leggett 1988).

Finally, I am concerned that the manner in which the $\bar{F}$ parameter in the Eggers model is usually defined (as the mean level of food in stomachs throughout the estimation period) may lead some to compute this parameter as a grand mean of food weights across all fish collected or as some weighted mean of means. Distributions of food weights among fish from a single collection are often positively skewed, not only for yellow perch (Hayward et al. 1989) but for other species (Cochran and Adelman 1982). Using means to describe central tendencies of food weights within individual collections, therefore, will tend to overestimate daily ration. Either medians should be used as point estimates of food weights for individual collections (as in this study), or a normalizing transformation of the data should be made (Boisclair and Leggett 1988).

I have shown here that the previously known lack-of-closure bias associated with use of the Eggers model (Eggers 1979) can be important for estimates of yellow perch daily rations. Moreover, a previously unreported and distinct bias which increases with daily ration level as well as with the within-day sampling interval has been indicated. Additional bias may be incurred when $\bar{F}$ in the Eggers model is computed without regard to the skewness typically present in distributions of food weights for a given collection of fish. While important bias can result from any one of these three sources, the possibility exists for even more accentuated bias arising from multiple sources. The previously known bias can be corrected for as shown, as can the skewness in food weight distributions. Caution is advised, however, when using the Eggers model and within-day sampling interval exceeding 3 h, particularly when daily rations are in the middle to high range for a given fish species.

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References

ANGRADI, T. R., AND J. S. GRIFFITH. 1990. Diel feeding chronology and diet selection of rainbow trout (*Oncorhynchus mykiss*) in the Henry's Fork of the Snake River, Idaho. Can. J. Fish. Aquat. Sci. 47: 199–209.

BAJKOV, A. D. 1935. How to estimate the daily food consumption of fish under natural conditions. Trans. Am. Fish. Soc. 65: 288–289.

BOISCLAIR, D., AND W. C. LEGGETT. 1988. An in situ experimental evaluation of the Elliott Persson and the Eggers models for estimating fish daily ration. Can. J. Fish. Aquat. Sci. 45: 138–145.

1989. Among population variability of fish growth: I. Influence of the quantity of food consumed. Can. J. Fish. Aquat. Sci. 46: 457–467.

COCHRAN, P. A. 1979. Comments on some recent methods for estimating food consumption by fish. J. Fish. Res. Board Can. 36: 1018.

COCHRAN, P. A., AND I. A. ADELMAN. 1982. Seasonal aspects of daily ration of largemouth bass (*Micropterus salmoides*) with an evaluation of gastric evacuation rates. Environ. Biol. Fishes 7: 265–275.

EGGERS, D. M. 1977. Factors in interpreting data obtained by diel sampling of fish stomachs. J. Fish. Res. Board Can. 34: 290–294.

1979. Comments on some recent methods for estimating food consumption by fish. J. Fish. Res. Board Can. 36: 1018–1019.

ELLIOTT, J. M., AND L. PERSSON. 1978. The estimation of daily rates of food consumption for fish. J. Anim. Ecol. 47: 977–991.

HAYWARD, R. S., AND F. J. MARGRAF. 1987. Eutrophication effects on prey size and food available to yellow perch in Lake Erie. Trans. Am. Fish. Soc. 116: 210–223.

1988. Ecology of yellow perch in Lake Erie. Commercial Fisheries Research and Development Project 3-379-R, Completion Report, Ohio Department of Natural Resources, Columbus, OH. 138 p.

HAYWARD, R. S., F. J. MARGRAF, C. T. KNIGHT, AND D. J. GLOMSKI. 1989. Gear bias in field estimation of the amount of food consumed by fish. Can. J. Fish. Aquat. Sci. 46: 874–876.

HEWETT, S. W., AND B. L. JOHNSON. 1987. A generalized bioenergetics model of fish growth for computers. University of Wisconsin Sea Grant Institute, Madison, WI.

KITCHELL, J. F., D. J. STEWART, AND D. WEININGER. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 34: 1922–1935.

PERSSON, L. 1979. The effects of temperature and different food organisms on the rate of gastric evacuation in perch (*Perca fluviatilis*). Freshwater Biol. 9: 99–104.

RUGGERONE, G. T. 1989. Gastric evacuation rates and daily ration of piscivorous coho salmon (*Oncorhynchus kisutch*) Walbaum. J. Fish Biol. 34: 451–463.

SAS INSTITUTE, INC. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute, Inc. Cary, NC.

Variation in Prevalence of *Nanophyetus salmincola*, a Parasite Tag Indicating U.S. Northwest Origin, in Steelhead Trout (*Oncorhynchus mykiss*) Caught in the Central North Pacific Ocean

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Dalton, T. J. 1991. Variation in prevalence of *Nanophyetus salmincola*, a parasite tag indicating U.S. Northwest origin, in steelhead trout (*Oncorhynchus mykiss*) caught in the central North Pacific Ocean. Can. J. Fish. Aquat. Sci. 48: 1104–1108.

Infections with the parasite "tag" *Nanophyetus salmincola* indicated that in 1986 and 1987, steelhead trout (*Oncorhynchus mykiss*) from the U.S. Northwest (Washington to northern California, including Idaho) were relatively abundant in the central North Pacific Ocean as far west as 165°E, about 5000–5500 km from their area of origin. Infection prevalence (the frequency of the presence of infection) was estimated annually for specimens stratified by 5°-longitude areas. East–west and north–south trends in prevalence occurred in 1986 but not in 1987. Correlation analysis indicated no between-year correlation in the relative magnitude of prevalence in areas sampled in both 1986 and 1987 ($r = 0.017$). Confidence intervals (90%) for overall annual prevalence in the central North Pacific Ocean steelhead population were 29.9–61.3% (point estimate = 45.4%) for 1986 and 43.3–63.0% (point estimate = 53.2%) for 1987.

L'étude des infections au parasite "marqueur" *Nanophyetus salmincola* a révélé qu'en 1986 et 1987, la truite arc-en-ciel (*Oncorhynchus mykiss*) du nord-ouest des États-Unis (de l'État de Washington jusqu'au nord de la Californie, y compris l'Idaho) était relativement abondante dans le centre du Pacifique nord et se retrouvant jusqu'au 165° de longitude E, soit à quelque 5 000 – 5 500 km de son aire d'origine. On a estimé pour chaque année la prévalence de l'infection (fréquence de l'infection) d'après des spécimens répartis en zones de 5° de longitude. On a constaté que la prévalence suivait des tendances d'est en ouest et du nord au sud en 1986, mais non en 1987. L'analyse de corrélation n'a révélé aucune corrélation entre les années au point de vue de l'importance relative de la prévalence dans les zones échantillonnées tant en 1986 qu'en 1987 ($r = 0{,}017$). Les intervalles de confiance (90 %) s'appliquant à la prévalence annuelle générale dans le centre du Pacifique nord étaient de 29,9–61,3 % (45,5 % en estimation ponctuelle) pour 1986 et de 43,3–63,0 % (53,2 % en estimation ponctuelle) pour 1987.

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Anadromous rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), known as steelhead trout, are found widely in Pacific drainages of North America from central California to the south side of the Alaska peninsula (Sutherland 1973). Until recently, *O. mykiss* in Asia were known as *Salmo mykiss*, the Kamchatka trout, and anadromous populations are found on the Kamchatka Peninsula, occasionally in Northern Okhotsk Sea drainages, and perhaps in the Komander Islands (Margolis 1984). Steelhead are distributed across the Pacific Ocean north of about 39°N (but only rarely north of the Aleutian Island chain) during the spring and summer; the southern boundary shifts northward as the season (April–September) progresses (Sutherland 1973; Okazaki 1983). The Asian form probably represents only a small proportion of the total population, and the oceanic distribution of these is poorly known (Harris 1988). Tagging studies have demonstrated that *O. mykiss* from North America are distributed at least as far west as 162°28′E and as far south as 40°58′N and that stocks originating from Alaska to California are widely found in this range east of 167°E (Light et al. 1988). However, the relative abundances of these stocks over this large ocean range are not well known.

Margolis (1984, 1985) used the presence of the freshwater trematode parasite *Nanophyetus salmincola* in ocean-caught steelhead trout to indicate origin from the "U.S. Northwest" (Washington to northern California, including Idaho). The parasite is acquired by juvenile steelhead only in freshwater but persists throughout all or most of the sea life of the host. The freshwater range in which steelhead can become infected is determined by the range of the obligatory first intermediate host of these parasites, snails of the genus *Juga*. Although *N. salmincola* occurs in Asia, it is sympatric with *O. mykiss* only in the U.S. Northwest. Also, *N. salmincola* exhibits site selectivity, concentrating most abundantly in the kidney (Margolis 1984). In this study, the kidneys of steelhead caught in the central North Pacific Ocean during June–July of 1986 and 1987 were examined for *N. salmincola*, and annual infection prevalence (the frequency of the presence of infection) was estimated for 5°-longitude areas of this ocean region. Comparisons of prevalence among areas were made both within and between years, and confidence intervals and point estimates were made for overall prevalence in the steelhead population of the entire ocean region.

Materials and Methods

As a part of the cooperative program of the International North Pacific Fisheries Commission (INPFC), the Fisheries Agency of Japan (FAJ) supplied to the Fisheries Research Institute (FRI) samples of steelhead that were caught in the central North Pacific Ocean in 1986 and 1987. The steelhead were caught with gill nets and longlines by Japanese research vessels and mothership fishery vessels. Weight, length, sex, and gonad weight were recorded for each fish onboard the vessel, and a scale sample was taken for age determination. The posterior one third of the kidney was also removed from 1987 specimens. The carcasses or kidney samples were then immediately frozen, and after temporary storage in Japan they were shipped frozen to FRI. There, the 1986 carcasses were thawed and kidney samples were removed and refrozen. Whole kidneys were removed from about one third of the 1986 steelhead, but only the posterior one third of the kidney was removed from the rest, as had been done with the 1987 steelhead. The parasites concentrate very heavily in this portion (Donham 1928; Bennington and Pratt 1960; Millemann and Knapp 1970; Wood 1974), so only this portion was removed to save time in the examinations. Examinations were performed on the kidneys of 311 and 211 steelhead caught in 1986 and 1987, respectively. The fish were caught within the following ranges: 1986, 167°31′E to 177°30′W and 50°40′N to 42°29′N; 1987, 166°30′E to 173°28′W and 49°23′N to 41°30′N.

Frozen kidney samples were homogenized prior to examination by repeated and alternated mincing (with a heavy knife) and stirring (with a glass slide) of the samples on a Plexiglas cutting board. Aliquots of this homogenate were squash-mounted between glass plates (20.3 × 10.2 × 0.3 cm). Homogenized kidney tissue squashes into a thin layer that can be thoroughly examined without difficulty. All specimens were examined under high-intensity transmitted light with a 7–60× binocular dissecting microscope.

Owing to time constraints, it was not feasible to examine all of a kidney sample, even when this was only the posterior one third of the kidney. One-quarter sample aliquots of a homogenized kidney sample, measured by weight or volumetrically after transfer of the homogenate to a volumetric syringe, were sequentially examined until infection was found or two quarters had been thoroughly examined. An examination ceased upon the positive identification of infection. To confirm the infection, I normally would search until more than one parasite (metacercarial cyst) was located. At the most, only one half of a kidney sample was ever examined. Since the sample was homogenized, one half of the posterior one third of the kidney was examinated regardless of whether all of the kidney had been removed or just the posterior one third. Time was taken as needed for thorough examination of the aliquots, so whole kidneys required lengthier examinations.

Since only as much as one half of a sample was ever examined, it seemed likely that some light infections (those involving only one or a few cysts) would be overlooked. One-quarter sample aliquots were sequentially examined, rather than simply examining one half of a sample, so that the number of overlooked infections in groups of specimens could be estimated by the method described below. Fish were designated as "infected" or as "negative for infection," rather than "uninfected," since infection may have been missed.

The kidney examination results were grouped as follows:

Category	First quarter	Second quarter	No. of fish
1	Infection found (+)	Not examined	I
2	No infection found (−)	Infection found (+)	i
3	No infection found (−)	No infection found (−)	U

The number of fish in which infection was found (n) equals $I + i$, and the total number of fish examined (N) equals $I + i + U$. To estimate prevalence it was necessary to have an estimate of the number of fish in Category 3 that were actually infected. For any individual specimen in the sample, the best estimate of the probability of finding infection in a quarter given that infection had not been found in another quarter ($P(+/-)$) was

$$P(+/-) = i/(i + U).$$

This proportion was used to estimate the probability that infection would be found in either of the two quarters of the kidney sample that were not examined for fish in category 3 ($P(U_i)$):

$$P(U_i) = P(+/-) + P(+/-) - [P(+/-) \times P(+/-)].$$

$P(U_i)$ was then multiplied by the number of fish in category 3 (U) to estimate the number of fish in this category that were actually infected (U_i):

$$P(U_i) \times U = U_i.$$

This equation yielded a nonwhole number estimate of U_i. This was rounded to the nearest whole number before being added to the number of specimens in which infection was actually observed (n) to yield an estimate of the actual number of infected fish in the sample (n_a):

$$n + U_i \text{ (rounded off)} = n_a.$$

The estimate of prevalence (Pr) for the sample was

$$Pr = n_a/N.$$

This method was used to estimate annual prevalence in the 5°-longitude areas. The areas, except area 16, have previously been used for analysis of data from he Japanese high-seas salmon fisheries (Fredin and Worlund 1974; Myers et al. 1984; Harris 1988). Area 16 was constructed to be of comparable size with the others and to include all other locations where steelhead were caught. To examine spatial trends in the relative abundance of infected fish, within-year homogeneity of prevalence frequencies among these areas was tested by chi-square analysis using a significance level of $p = 0.05$, with Cochran's continuity correction method employed as needed. Larger scale spatial trends were examined by combining prevalence frequencies for selected areas and treating the combined frequency as a single prevalence frequency for the ocean region encompassed by the combined areas. Such a combined frequency could then be compared with other prevalence frequencies by chi-square analysis.

Results

Estimated prevalence ranged from 22.9 to 78.6% in 1986 and from 25.0 to 75.0% in 1987 (Tables 1 and 2; Fig. 1). These prevalences demonstrate that U.S. Northwest origin steelhead

TABLE 1. *Nanophyetus salmincola* examination results for steelhead stratified by 5°-longitude areas (number of specimens examined (*N*), number of specimens in which infection was observed (*n*), number of specimens in which infection was observed in the first one-quarter aliquot (*I*), number of specimens in which infection was observed in the second one-quarter aliquot but not the first (*i*), number of specimens in which infection was not observed (*U*), estimated number of infected specimens (n_a)).

	1986						1987					
Area	*N*	*n*	*I*	*i*	*U*	n_a	*N*	*n*	*I*	*i*	*U*	n_a
5	163	70	65	5	93	79	12	6	6	0	6	6
7	15	6	6	0	9	6	12	7	6	1	5	9
9	14	10	9	1	4	11	28	14	13	1	14	16
12	13	5	5	0	8	5	4	1	1	0	3	1
13	35	6	5	1	29	8	23	10	7	3	13	14
14	—	—	—	—	—	—	36	14	12	2	22	18
15	71	24	20	4	47	31	62	26	24	2	36	30
16	—	—	—	—	—	—	34	17	15	2	17	20

TABLE 2. *Nanophyetus salmincola* prevalences (*Pr*) and arc-sine transformed prevalences ($Pr_{a\text{-}s}$) in steelhead stratified by 5°-longitude areas.

	1986		1987	
Area	*Pr* (%)	$Pr_{a\text{-}s}$ (%)	*Pr* (%)	$Pr_{a\text{-}s}$ (%)
5	48.5	44.140	50.0	45.0
7	40.0	39.232	75.0	60.0
9	78.6	62.445	57.1	49.082
12	38.5	38.351	25.0	30.0
13	22.9	28.590	60.9	51.296
14	—	—	50.0	45.0
15	43.7	41.381	48.4	44.083
16	—	—	58.9	50.127

made up large or sometimes predominant proportions of the steelhead present in the sampled areas.

There were significant differences among the prevalence frequencies in areas sampled in 1986 (p = 0.006). Spatial prevalence trends were best analyzed by grouping the areas by position north or south of 46°N latitude. For both the northern and southern groups, prevalence was greatest in the most easterly area (areas 9 and 15, both located east of 180°). However, the differences among the prevalance frequencies for the areas of each group were not significant (north: p = 0.0074; south: p = 0.119). Prevalences in areas north of 46°N were mostly greater than prevalences in areas south of this line. The area prevalence frequencies within the groups were combined (north: 96/192 (50.0%); south: 42/119 (35.3%)) and the combined frequencies were compared; there was a significant difference between the combined frequencies (p = 0.026), indicating that infected steelhead had higher relative abundance north of 46°N than south of this line.

There were no apparent trends, either on a north–south or east–west basis, in the area prevalences for 1987, and a comparison of these area prevalence frequencies did not indicate significant differences among them (p = 0.608). The areas were grouped by position north or south of 46°N; within-group comparisons of the area prevalence frequencies indicated no significant within-group differences in the frequencies (north: p = 0.441; south: p = 0.578). A comparison of the combined frequencies for the north and the south groups indicated no significant difference between these combined frequencies (p = 0.446). Apparently, the relative abundance of infected steelhead did not differ significantly among the areas sampled in 1987.

Pairwise chi-square comparisons (with Cochran's continuity correction) of 1986 and 1987 prevalence frequencies for the six areas sampled in both years (Table 2) indicated significant difference at the 0.05 level between only the annual frequencies in area 13 (p = 0.006). However, for several of these areas, sample sizes were rather small in at least one of the years. The paired annual percentage prevalences for these areas were analyzed for correlation after the prevalences were normalized by arc-sine transformation (Table 2). There was no between-year correlation in the relative magnitude of prevalence in these years (r = 0.017).

The 1986 and 1987 samples were collected over approximately the same large region of the central North Pacific Ocean. To make confidence intervals (90%) and point estimates of annual prevalence over this region, the area prevalences (transformed) were treated as independent estimates of prevalence in the overall population. These point estimates were preferable to the overall prevalence in all of a year's samples; because prevalence varied among areas, overall prevalences would be biased by large sample sizes in particular areas (e.g. 52.4% of all 1986 sepcimens were captured in area 5). The point estimates of overall prevalence were compared for equality using a t-test on the transformed area prevalences. Such a test considered area prevalence variations and made no assumption that the overall population would be well represented by a total sample (all yearly specimens). For 1986, the 90% confidence interval for overall prevalence was 29.9–61.3%, and the point estimate was 45.4%; for 1987, the 90% confidence interval was 43.3–63.0%, and the point estimate was 53.2%. The point estimates differed by only 7.8%, and the t-test did not indicate that prevalence differed between years.

Discussion

Dalton (1989a) reported that in 1985, steelhead caught in the central North Pacific Ocean east of 180° had significantly greater *N. salmincola* prevalence than steelhead caught west of that line (p = 0.003), a trend that was not repeated to a significant extent in 1986 or 1987. Over the years 1985–87, infected steelhead showed no consistency in their year-to-year relative abundance among areas of the central North Pacific Ocean. It is important to note, however, that prevalence might not be a consistent index of the relative abundance of U.S.

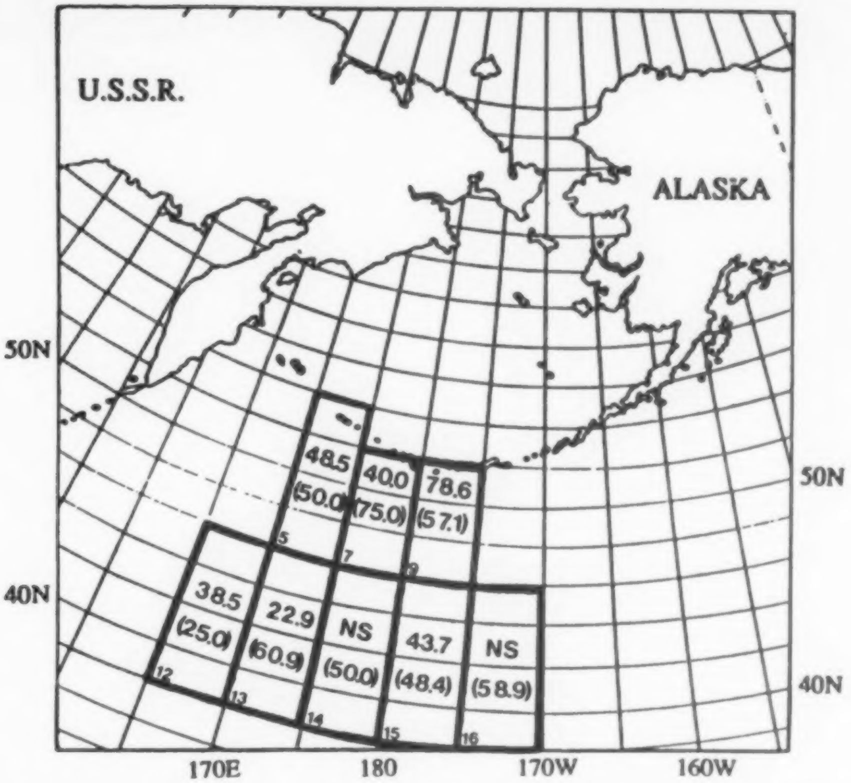

FIG. 1. Percentage prevalence of *N. salmincola* in 1986 and 1987 (in parentheses) specimens stratified by 5°-longitude areas (NS = no sample).

Northwest origin steelhead among ocean areas if there are largely or wholly uninfected U.S. Northwest stocks with different ocean distribution patterns than stocks that have high prevalence. In this case, the ratio of infected to uninfected U.S. Northwest steelhead in an ocean area would depend on which U.S. Northwest stocks contributed most to that area. Dalton (1989b) reported that prevalence in emigrating steelhead smolts in 1987 varied widely among regions of the U.S. Northwest. Furthermore, annual prevalence in an ocean area sample may be influenced by the sampling dates and precise locations of capture within the area because steelhead as a group migrate northward as the summer progresses (Sutherland 1973; Okazaki 1983). Finally, the year-class composition of an area sample may affect prevalence because prevalence may inherently vary among year-classes. Sample sizes did not permit further stratification by location of capture, date of capture, and year-class.

Compared with the 1986 and 1987 point estimates of overall annual prevalence, the grand annual prevalences reported for steelhead caught in the central North Pacific Ocean during 1983 (Margolis 1984), 1984 (Margolis 1985; Dalton 1989a), and 1985 (Dalton 1989a) were low or very low (Table 3). Dalton (1989a) reasoned that annual variations in prevalence are likely to occur owing to annual fluctuations in both the parasite's freshwater abundance and the annual relative abundance at sea of the various steelhead populations and stocks. Most fish parasite populations are basically unstable (Kennedy 1977), and very large annual fluctuations in the abundance of steelhead stocks are common (Light 1987). However, Dalton also suggested that the annual prealence variations could in part be due to spatial and temporal differences in sampling effort among years and variations in the examination protocols.

TABLE 3. *Nanophyetus salmincola* prevalences for entire sampled ocean regions, 1983–87.

Year	Report	Prevalence (%)	Sampling months
1983	Margolis 1984	10.2	April–July
1984	Margolis 1985	18.5	April–July
1984	Dalton 1989a	26.5	June–July
1985	Dalton 1989a	36.1	June–July
1986	Present study	45.4	June–July
1987	Present study	53.2	June–July

Based on *N. salmincola* infection levels in freshwater juvenile salmonids collected in 1987 and 1988, Dalton (1989b) made a qualified estimate that only about 60% of U.S. Northwest steelhead returning from sea were infected. If this figure is accurate, then the total proportion of U.S. Northwest steelhead in the ocean-caught samples must be substantially higher than prevalence (the proportion of infected steelhead) because these samples could be expected to contain both infected and uninfected U.S. Northwest steelhead. Finally, it is apparent that U.S. Northwest steelhead were relatively abundant as far west as 165°E during the summers of 1986–87, about 5000–5500 km from their freshwater areas of origin. The Japanese high-seas salmon fisheries operate west of 174°E and south of 46°N in the spring and summer. U.S. Northwest origin steelhead no doubt made up a substantial proportion of the steelhead bycatch, which numbered at least 8429 in 1986 (FAJ, Tokyo, Japan, unpubl. data) and 7718 in 1987 (FAJ, Tokyo, Japan, unpubl. data).

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References

Bennington, E. E., and I. Pratt. 1960. The life history of the salmon-poisoning fluke, *Nanophyetus salmincola* (Chapin). J. Parasitol. 46: 91–100.

Dalton, T. J. 1989a. Parasite tag identifications of U.S. Pacific Northwest origin steelhead trout caught in the North Pacific Ocean, 1984–1987. (Document submitted to the Int. N. Pac. Fish. Comm. (FRI-UW-8905); available from Fisheries Research Institute, University of Washington, Seattle, WA 98195, USA. 52 p.)

1989b. The use of a freshwater trematode as a parasite tag to indicate continental region of origin of ocean-caught steelhead trout. M.S. thesis, University of Washington, Seattle, WA.

Donham, C. R. 1928. Salmon poisoning in dogs. Ph.D. thesis, Oregon State University, Corvallis, OR.

Fredin, R. A., and D. D. Worlund. 1974. Catches of sockeye salmon of Bristol Bay origin by the Japanese mothership salmon fishery, 1956–70. Int. N. Pac. Fish. Comm. Bull. 30: 1–80.

Harris, C. K. 1988. Recent changes in the pattern of catch of North American salmonids by the Japanese high seas salmon fisheries, p. 41–65. *In* W. McNeil [ed.] Salmon production, management, and allocation: biological, economic and policy issues. Proc. World Salmonid Conference, Oct. 1986. Oregon State University Press, Corvallis, OR.

Kennedy, C. R. 1977. The regulation of fish parasite populations, p. 63–110. *In* G. W. Esch [ed.] Regulation of parasite populations. Academic Press, New York, NY.

Light, J. T. 1987. Coastwide abundance of North American steelhead trout. (Document submitted to annual meeting of the Int. N. Pac. Fish. Comm., 1987 (FRI-UW-8710); available from Fisheries Research Institute, University of Washington, Seattle, WA 98195, USA. 18 p.)

Light, J. T., S. Fowler, and M. L. Dahlberg. 1988. High seas distribution of North American steelhead as evidenced by recoveries of marked or tagged fish (Document submitted to the Int. N. Pac. Fish. Comm. (FRI-UW-8816); available from Fisheries Research Institute, University of Washington, Seattle, WA 98195, USA. 21 p.)

Margolis, L. 1984. Preliminary report on identification of continent of origin of ocean-caught steelhead trout, *Salmo gairdneri*, using naturally occurring parasite "tags." (Available from Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada. 23 p.)

1985. Continent of origin of steelhead, *Salmo gairdneri*, taken in the North Pacific Ocean in 1984, as determined by naturally occurring parasite "tags." (Document submitted to the Int. N. Pac. Fish. Comm., Tokyo, Japan, October 1985; available from Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada. 18 p.

Millemann, R. E., and S. E. Knapp. 1970. Biology of *Nanophyetus salmincola* and "salmon poisoning" disease. Adv. Parasitol. 8: 1–41.

Myers, K. W., D. E. Rogers, C. K. Harris, C. M. Knudsen, R.V. Walker, and N. D. Davis. 1984. Origins of chinook salmon in the area of the Japanese mothership and landbased driftnet salmon fisheries in 1975–1981. (Document submitted to annual meeting of the Int. N. Pac. Fish. Comm., 1984; available from Fisheries Research Institute, University of Washington, Seattle, WA 98195, USA. 208 p.)

Okazaki, T. 1983. Distribution and seasonal abundance of *Salmo gairdneri* and *Salmo mykiss* in the North Pacific Ocean. Jpn. J. Ichthyol. 30: 235–246.

Sutherland, D. F. 1973. Distribution, seasonal abundance, and some biological features of steelhead trout, *Salmo gairdneri*, in the North Pacific Ocean. Fish. Bull. 71: 787–807.

Wood, J. W. 1974. Diseases of Pacific salmon: their prevention and treatment. (Publ. No. 49: 18–31; available from Washington Department of Fisheries, Olympia, WA.)

Hatchery Growth of Pure Strains and Intraspecific Hybrids of Juvenile Arctic Charr, *Salvelinus alpinus* (Canadian × Norwegian Charr)

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de March, B. G. E. 1991. Hatchery growth of pure strains and intraspecific hybrids of juvenile Arctic charr, *Salvelinus alpinus* (Canadian × Norwegian charr). Can. J. Fish. Aquat. Sci. 48: 1109–1116.

Growth parameters of 36 tank populations representing 23 full-sib families of Nauyuk and Norwegian Arctic charr (*Salvelinus alpinus*) and both reciprocal hybrids were determined for 1 yr. Hatching success of families was a maternal characteristic. Mean weights of families at 30 d after swim-up were related to maternal effects as well as the general and specific combining abilities of the two strains, with the weight contribution of the Nauyuk parent(s) larger than that of the Norwegian. At 125 d after swim-up, the mean weights and coefficients of variation (CVs) in the four types of crosses were Nauyuk dam × Nauyuk sire, 2.05 g ± 20%; Nauyuk dam × Norwegian sire, 2.10 g ± 24%; Norwegian dam × Nauyuk sire, 1.91 g ± 26%; and Norwegian dam × Norwegian sire, 1.71 g ± 31%. After 125 d, the Nauyuk dam × Norwegian sire families showed superior growth, while the Norwegian dam × Nauyuk sire families grew slowly and developed large size variation. At 325 d, reciprocal effects were evident, and mean weights and CVs were Nauyuk dam × Nauyuk sire, 36 g ± 45%; Nauyuk dam × Norwegian sire, 43 g ± 48%; Norwegian dam × Nauyuk sire, 30 g ± 68%; and Norwegian dam × Norwegian sire, 38 g ± 36%. The largest individual fish in the experiment were hybrids.

On a mesuré pendant 1 an les paramètres de croissance de 36 populations d'ombles chevaliers (*Salvelinus alpinus*) gardées en bassin et représentant 23 familles de Nauyuk et de Norvégiens et des deux types d'hybrides réciproques en fratrie. L'éclosabilité dans les familles était une caractéristique maternelle. Le poids moyen par famille, 30 jours après la remontée était lié aux effets maternels ainsi qu'aux aptitudes conjointes générales et particulières des deux souches, la contribution pondérale des parents Nauyuk étant plus importante que celle des Norvégiens. Cent vingt-cinq jours après la remontée, le poids moyen et le coefficient de variation (CV) des quatre types de croisements avaient les valeurs suivantes: femelle Nauyuk × mâle Nauyuk, 2,05 g ± 20 %; femelle Nauyuk × mâle Norvégien, 2,10 g ± 24 %; femelle Norvégien × mâle Nauyuk, 1,91 g ± 26 %; et femelle Norvégien × mâle Norvégien, 1,71 g ± 31 %. Au bout de 125 jours, la croissance des poissons des familles produites par le croisement femelle Nauyuk × mâle Norvégien était supérieure, tandis que celle des familles issues du croisement femelle Norvégien × mâle Nauyuk était lente, ces dernières familles présentant en outre d'importantes variations de taille. À 325 jours, les effets réciproques étaient manifestes; le poids moyen et le CV avaient les valeurs suivantes : femelle Nauyuk × mâle Nauyuk, 36 g ± 45 %; femelle Nauyuk × mâle Norvégien, 43 g ± 48 %; femelle Norvégien × mâle Nauyuk, 30 g ± 68 %; et femelle Norvégien × mâle Norvégien, 38 g ± 36 %. Les poissons les plus gros de l'expérience étaient des hybrides.

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In aquaculture, intra- and interspecific hybrids of fish species are sometimes preferred over pure or inbred strains if they show superior performance or a desirable combination of traits. Bakos (1987) cited numerous cases of hybrid vigour for characters with low heritability, such as survivorship, occurring in intraspecific crosses of warmwater species. More evidence comes from crosses among cultured strains of carp (*Cyprinus carpio*), where hybrids performed better than their parents (Bakos 1979; Suzuki 1976; Moav et al. 1974). Results for other species and for interspecific crosses are more variable (summaries in Refstie 1983; Chevassus 1979; Bakos 1987; Naevdal and Dalpadado 1987; Kraznai 1987). Hybrid vigour has been reported in crosses of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) (Webster and Flick 1981; Gall 1969; Ayles and Baker 1983). Hybrids often combine different desirable characteristics of both parents. For example, hybrids between brook and lake trout (*Salvelinus namaycush*) have been shown to combine the fast growth and early maturation of the brook trout with the ability of the lake trout to survive well in deep cold-water lakes (Ayles 1974). Hybrids between Dolly Varden (*Salvelinus pluvius*) and brook trout combine fast growth rate and good survival (Kato 1966). Refstie (1983) concluded that, in general, heterosis is of little importance for economically important traits in salmonids.

In this study the growth characteristics of two pure and relatively unselected strains of Arctic charr[1] (*Salvelinus alpinus*), namely Canadian and Norwegian charr, and their hybrids were compared. Arctic charr have been reared at the Rockwood Aquaculture Research Centre at Gunton, Manitoba, since 1980 and have demonstrated potential as an aquaculture species (Papst and Hopky 1984; Baker 1983). The species' most desir-

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[1] Recommended spelling is "char" (*A List of Common and Scientific Names of Fishes from the United States and Canada*. 1980. 4th ed. Spec. Publ. No. 12, American Fisheries Society, Bethesda, MD).

TABLE 1. Chronology of measured and estimated characters. Abbreviations for strains and crosses are given in the text. Wt30d is the measured mean weight in a family at 30 d; k, t_0, A, Wt125, Wt250, and Wt325 (weights at 125, 250, and 325 d) are parameters and estimates from Equation (1); CV125 and CV325 are the estimated CVs for 125 and 325 d from Equations (1) and (2) for each tank; Wt13cm and Wt17cm are the expected weights at lengths of 13 and 17 cm in Survey 3 obtained from regressions for each tank.

Date	Event	Characters
Fall 1980	Eggs for parents collected	
Jan. 1981	Parents hatched	
Sept. 11, 1986	NAFs spawned and crossed with NAMs and NOMs	
Sept. 30	NOFs spawned and crossed with the same NAMs and NOMs	
Dec. 4–9	AAs and AOs hatched	% hatch
Dec. 14–19	OAs and OOs hatched	% hatch
Jan. 8, 1987	AAs and AOs transferred to 61-L tanks at 8°C	
Jan. 28	OAs and OOs transferred to 61-L tanks at 8°C	
Feb. 6	AAs and AOs batch weight taken	Wt30d
Feb. 24	OAs and OOs batch weight taken	% hatch
Mar. 24	Families of 110 or 220 fish/tank set up at 9.5°C	
Mar. 24, 1987, to Mar. 9, 1988	Batch weights taken at 2- to 4-wk intervals	A, k, t_0, Wt125d, Wt250d, Wt325d
Apr. 21 to June 23, 1987	Survey 1. Fish weighed and measured individually	CV125d, CV250d, CV325d, Wt13cm, Wt17cm
Aug. 4 to Oct. 15, 1987	Survey 2. Fish weighed and measured individually	
Nov. 15, 1987, to Mar. 9, 1988	Survey 3. Fish weighed and measured individually	
Jan. 12 to Mar. 9, 1988	Last batch weights taken	

able characteristics are its hardiness, ability to prosper at high densities, attractive appearance, and relatively rapid growth at temperatures below 15°C. Lack of synchrony in maturation, low fertilization rates, and large size variability of offspring still remain undesirable characteristics. Baker (1983) demonstrated that charr from the Fraser River in Labrador, Canada, grew faster than those from Norway at temperatures >14°C, but grew similarly at 7 and 10°C. Norwegian charr have a smaller adult size, smaller egg size (~4 rather than ~5 mm), and generally lower juvenile survival than Canadian strains (Krieger 1987). However, females ripen more synchronously than females of Canadian strains, in which egg ripening is sometimes spread over 3 mo. The Norwegian strain has an attractive dark pigmentation and does not tend to take on the monstrous appearance that some of the Canadian strains do. The examination of F1 hybrids was merited with this combination of desirable and undesirable aquaculture characteristics of both strains.

Methods

The Canadian parental fish were the first laboratory generation, originally obtained as artificially fertilized eggs of an anadromous stock from Nauyuk Lake (68°22′N, 107°43′E) on the Kent Peninsula, Northwest Territories, Canada, in 1980. The Norwegian parental fish were the first laboratory generation of an anadromous strain from Storvatn in northern Norway (68°10′N, 16°30′E) and were received as eyed eggs from the Sunndalsora Hatchery, Sunndalsora, Norway, in December 1980. Neither strain was maintained as pedigreed fish, so it is not known whether parents within strains were related.

The following abbreviations will be used in this paper: Nauyuk female or dam = NAF, Nauyuk male or sire = NAM, Norwegian female or dam = NOF, and Norwegian male or sire = NOM. The four types of offspring are designated as follows: NAF × NAM = AA, NAF × NOM = AO, NOF × NAM = OA, and NOF × NOM = OO.

The chronology of this experiment is given in Table 1. Since all batch weights (the combined weights of all fish in a tank) and individual fish measurements could not be taken on the same date or at the same age because of the volume of data, choices about which tanks (families) to measure on a date were made either by randomization or randomization within a rotation of all tanks and by sampling near predetermined mean weights. Then, model parameters and expected values obtained from curves describing temporal events, usually fitted with a very high degree of predictability, were used to summarize differences between tanks.

Individual NAFs were stripped of eggs on September 11, 1986. Half of the eggs from each female were fertilized with milt from an NAM and the other half with milt from an NOM (Table 1). NOFs were stripped on September 30, the eggs divided into two or four batches, and these were fertilized with milt from males previously chosen for one NAF. The main repeated experimental unit is thus a "mating set," ideally a factorial mating design of m sires × n dams, with identified dams and sires of both strains. Initially, 9 NAFs and 10 NOFs were crossed, resulting in the design in Table 2. Some crosses either failed or resulted in insufficient offspring to continue. The final design for the examination of growth characters with five mating sets is given in Table 3.

Eggs were incubated at 6°C. Offspring from NAFs hatched between December 4 and 9 and those from NOFs between December 14 and 19 (Table 1). The juveniles were transferred to 61-L tanks on January 8 and 28, approximately 120 d after spawning and near swim-up. Each tank contained between 28 and 900 fish. Juvenile were reared at 8.0°C until March 24, when tanks with 110 and 220 fish were established for families with sufficient numbers. Crowding was not believed to strongly

TABLE 2. Percent hatch and mean weight of juveniles in grams at 30 d for all families. Parents are individually numbered. See text for abbreviations.

Mating set	Parents	% hatch	Wt30d
1	NAF4 NAM4	93	0.167
1	NAF4 NOM4	92	0.144
1	NOF7 NAM4	0	—
1	NOF7 NOM4	65	0.0965
2	NAF1 NAM1	66	0.179
2	NAF1 NOM1	68	0.165
2	NOF1 NAM1	32	0.199
2	NOF2 NAM1	16	0.133
2	NOF1 NOM1	88	0.0879
2	NOF2 NOM1	84	0.0920
3	NAF5 NAM5	53	0.225
3	NAF5 NOM5	85	0.190
3	4 crosses	0	—
	(2 NOFS each mated with NOM5 and NAM5)		
4	NAF6 NAM6	18	0.192
4	NAF6 NOM6	3	0.209
4	NOF8 NAM6	2	0.172
4	NOF8 NOM6	14	0.105
5	NAF3 NAM3	9	0.161
5	NAF3 NOM3	7	0.151
5	NOF5 NAM3	14	0.0853
5	NOF6 NAM3	80	0.111
5	NOF5 NOM3	68	0.0670
5	NOF6 NOM3	87	0.0563
6	NAF2 NAM2	24	0.151
6	NAF2 NOM2	69	0.134
6	NOF3 NAM2	74	0.123
6	NOF4 NAM2	49	0.114
6	NOF3 NOM2	86	0.107
6	NOF4 NOM2	45	0.0775
7, 8, 9	6 crosses	0	—
	(3 NAFS each mated with 1 NOM and 1 NAM)		

affect growth at these densities. Fish were reared at 9.5°C for the duration of the experiment (Table 1).

Fish were generously fed Bio-Diet starter diet (sizes 1 and 2) from swim-up and thereafter Martin Feed Mills Trout Feed grower diet at increasing granule and pellet sizes. The feed rate was between 100 and 150% of the amount suggested for rainbow trout (Leitritz and Lewis 1980). Water was treated using a biofiltration–recirculation system. Flow rates in the tanks were between 2 and 6 L/min, sufficient for maintaining oxygen saturation.

Measured and Estimated Growth Parameters

Thirty days after the transfer to the 61-L tanks, February 6 and February 24, 1987, samples of at least 75 random fish, or fewer if 75 were not available, were sampled from each of the 27 families to estimate the mean weights at 30 d (Tables 1 and 2).

Batch weights, that is the massed weight of all fish in the tank, and counts were taken in each of 36 tanks with fish from 23 families every 2–4 wk, until each mean weight was near 50 g, between January 12 and March 9, 1988 (Table 1). Intervals between measurements were chosen to be larger as the fish grew. Measurements in several tanks were ceased at a lower mean weight because growth was extremely slow and other measurements had already ceased. At least 18 batch weights were taken for each tank.

All growth curves of mean weight versus time were sigmoidal and could be described by the logistic growth curve (Ricker 1975)

$$(1)\quad \ln(\text{mean weight}) = \ln(A) - \ln(1+e^{-k(t-t_0)}) + \epsilon$$

in which t is the elapsed time after swim-up, A is the predicted asymptote of the mean weight, t_0 is the inflection point of the logistic curve, k is the instantaneous rate of growth at weight approaching zero, and ϵ is the residual error. The equation was fitted to all 36 growth curves using the nonlinear curve-fitting (NLIN) procedure in the SAS Statistical Package (SAS Institute Inc. 1985). Because NLIN produces only uncorrected sums of squares, R^2 values with correction for the mean were calculated manually as 1 − (sum of squares due to error)/(total sum of squares corrected for the mean). Predicted mean weights in each tank at 125, 250, and 325 d were calculated from these curves (Table 3).

All following linear analyses were done with the general linear models (GLM) procedure (SAS Institute Inc. 1985). In all models tested, Type IV sums of squares (i.e. partial (Draper 1966) or conditional sums of squares (Graybill 1967)) were used for tests of hypotheses.

Fish were weighed and measured individually three times: when the mean weight in the tank was near 1 g (Survey 1), near 10 g (Survey 2), and a third time when mean weight was near 50 g, or when the experiment was terminated (Survey 3) (Table 1). The following equation was fitted with the six statistics available from fish for each tank from the three surveys:

$$(2)\quad \ln(\text{variance}) = a\cdot\ln(\text{mean}) + b + \epsilon$$

in which a and b are regression parameters and ϵ is the model residual error for each tank. The predicted coefficients of variation (CV = standard deviation/mean) in each tank at 125 and 325 d were calculated from Equations (1) and (2) (Table 3).

The ln(weight) versus ln(length) regressions for Survey 3 were obtained from fish for all tanks after several conspicuous outliers due to spinal deformities were removed. There were significant differences among the slopes and intercepts of these regressions in spite of a high degree of overlap. It was felt that differences might be due to a small number of exceptionally large or small fish in some tanks. Therefore, differences in the condition were presented more conservatively as the predicted weights at 13 and 17 cm (Table 3) from each regression. This length range was well represented in all tanks.

Each one of the growth characters in Tables 2 and 3, that is hatching success, weight at 30 d, parameters of the logistic growth curve (A, k, and t_0), the predicted mean weights at 125, 250, and 325 d from Equation (1), the predicted CVs at 125 and 325 d from Equations (1) and (2), and the predicted weights at 13 and 17 cm, were described by the following model:

$$(3)\quad Y_{ijt} = \mu + f(n_t) + c_i + m_j + \epsilon_t$$

in which Y_{ijt} is a growth character for dam i and sire j in tank t from Table 2 or 3, μ is the common model mean, and ϵ_t is the tank residual error. The term $f(n_t)$ is the fitted linear combination $c\cdot n_t + d\cdot n_t^2$ describing the effects of n_t, the number of fish in a tank, c_i (i = 1, 4) described the mean effect of each of the four types of crosses (AA, AO, OA, and OO), and m_j (j = 1 to 5) described the mean effect of each mating set.

TABLE 3. Measured, estimated, and/or interpolated characters in all test tanks. See text and Table 1 for abbreviations. *n* is the number of fish per tank in Survey 3. The actual CVs measured in Surveys 1 and 3 and the actual range of weights measured in Survey 3 are shown in the right-hand columns.

		Parameters, estimates from Equation (1)							Equation (2)		Wt13cm, Wt17cm (g)		CVs, Surveys 1 and 3 (%)		Survey 3 weight (g)		
Mating set	Parents	*k*	t_0	*A*	*n*	Wt125	Wt250	Wt325	CV125 (%)	CV325					Min.	Mean	Max.
1	NAF4 NAM4	0.0179	322	56	215	1.62	12.2	28.9	23	52	22	51	24	64	5.9	51	146
2	NAF1 NAM1	0.0185	314	70	106	2.06	16.4	38.6	18	34	25	52	17	35	13.7	59	111
2	NAF1 NAM1	0.0185	310	65	211	2.05	16.1	37.0	20	35	21	51	20	38	8.0	53	97
3	NAF5 NAM5	0.0205	284	67	112	2.50	22.5	47.3	15	31	23	48	15	34	14.5	56	104
3	NAF5 NAM5	0.0185	301	68	218	2.48	18.9	41.2	16	33	21	47	15	37	6.2	55	120
5	NAF3 NAM3	0.0166	348	90	108	2.15	14.8	36.3	15	39	23	53	15	47	2.9	57	119
6	NAF2 NAM2	0.0176	348	107	101	2.08	16.2	42.6	23	53	23	54	22	54	7.1	48	129
6	NAF2 NAM2	0.0170	339	74	206	1.91	13.4	32.6	27	57	21	50	26	68	4.8	53	171
1	NAF4 NOM4	0.0172	345	76	218	1.69	12.5	31.7	28	63	21	50	27	72	4.5	55	198
2	NAF1 NOM1	0.0221	281	74	110	2.30	24.9	53.8	20	38	22	48	17	33	14.8	49	88
2	NAF1 NOM1	0.0209	278	55	211	2.15	19.7	40.3	21	40	21	47	20	37	3.1	43	89
3	NAF5 NOM5	0.0226	278	64	113	1.95	22.1	47.4	22	44	21	48	23	45	10.8	49	110
3	NAF5 NOM5	0.0220	279	57	220	1.86	19.8	42.2	23	40	21	49	23	40	12.1	45	101
5	NAF3 NOM3	0.0164	329	63	97	2.13	13.4	30.2	26	70	21	51	22	43	8.5	52	189
6	NAF2 NOM2	0.0237	265	76	105	2.64	31.3	61.4	23	34	21	47	21	31	5.4	54	102
6	NAF2 NOM2	0.0237	256	59	205	2.56	27.5	49.4	25	36	22	48	22	32	5.9	52	95
2	NOF1 NAM1	0.0179	312	71	107	2.40	17.5	39.5	23	37	21	47	22	40	13.1	54	134
2	NOF1 NAM1	0.0205	279	60	217	2.43	21.2	42.9	18	38	20	47	20	40	12.7	54	116
2	NOF2 NAM1	0.0136	357	54	103	2.21	10.2	21.2	27	85	22	51	21	91	5.4	44	245
2	NOF2 NAM1	0.0161	335	77	110	2.54	15.7	35.5	23	51	21	49	20	57	9.9	54	168
5	NOF5 NAM3	0.0158	319	32	94	1.44	8.1	16.8	40	139	21	—	41	166	3.7	30	210
5	NOF6 NAM3	0.0211	286	51	106	1.68	16.5	35.7	34	67	20	49	36	78	6.0	52	216
6	NOF3 NAM2	0.0159	360	72	111	1.67	10.6	26.0	24	78	22	53	27	103	6.3	50	246
6	NOF3 NAM2	0.0178	323	67	211	1.92	14.3	34.0	25	63	22	49	25	70	4.4	53	146
6	NOF4 NAM2	0.0206	286	53	208	1.87	17.2	36.8	22	55	21	49	24	70	5.5	55	182
1	NOF7 NOM4	0.0196	290	47	221	1.74	14.5	31.0	27	29	21	49	30	34	12.0	48	99
2	NOF1 NOM1	0.0221	283	65	105	1.92	21.2	46.7	36	33	20	47	35	31	6.3	58	98
2	NOF1 NOM1	0.0279	242	49	214	1.80	27.1	44.3	31	26	19	48	30	25	17.0	56	97
2	NOF2 NOM1	0.0234	270	56	111	1.79	21.4	44.0	33	30	18	45	33	30	12.3	54	103
2	NOF2 NOM1	0.0250	263	56	214	1.73	23.6	46.1	39	27	19	46	33	27	7.9	56	96
5	NOF5 NOM3	0.0180	330	51	99	1.25	9.8	24.6	39	46	21	50	33	54	4.7	49	126
5	NOF6 NOM3	0.0232	285	49	121	1.15	14.9	34.9	40	46	21	49	44	51	8.6	55	130
6	NOF3 NOM2	0.0256	235	49	104	2.82	29.6	45.1	25	33	21	49	23	32	11.0	61	109
6	NOF3 NOM2	0.0167	322	51	218	1.83	11.9	26.4	28	49	21	48	27	54	4.0	45	121
6	NOF4 NOM2	0.0265	251	55	106	1.90	27.3	48.3	29	36	23	52	28	35	13.9	59	128
6	NOF4 NOM2	0.0219	281	52	210	1.65	17.4	37.6	29	40	20	47	30	43	3.7	52	112

Expected mean values of characters in the four types of crosses were calculated using the LSMEANs least squares means (LSMEANS) option in PROC GLM (SAS Institute Inc. 1985 (Table 4). This linear estimation method holds the mating set and correction factor effects at the mean value to estimate predicted cross-type effects ($c_i + \mu$ from Equation (3)). The statistical significance of $f(n_t)$ was tested using the CONTRAST option in PROC GLM (SAS Institute Inc. 1985).

Subsamples from 34 of 36 families in this experiment were maintained for another year in an experiment with different objectives but also requiring uniform culture conditions. After that, only 14 families of pure strains were maintained.

Results

Tank Growth Characters

The 36 logistic growth curves describing the trend in the mean weight (Equation (1)) were fitted with a minimum R^2 of 0.988 and a mean R^2 of 0.995. Values of the parameters *A*, *k*, and t_0 for each curve are given in Table 3. The maximum mean square for error in the fitted equation was 0.032 and the average was 0.010. The least reliable predicted values of weight at given dates would have confidence limits of ±6% and the average ±2%. Thus there were usually some significant differences among predicted means in mating sets.

The linear regressions describing ln(variance) in terms of ln(mean) (Equation (2)) were fitted with high R^2 values in spite of only three points for each tank population. The mean R^2 value was 0.997, with 30 slopes significant at $P < 0.05$, five at $0.05 < P < 0.10$, and one at $P \sim 0.12$. Slopes ranged from 1.79 to 3.01 and intercepts from -1.78 to 4.12, and analysis of covariance showed numerous differences among them. The predicted CVs for 125 and 325 d are similar to the actual values in Surveys 1 and 3 (Table 3).

The R^2 values of the ln(weight) versus ln(length) regressions for fish in tanks in Survey 3 ranged from 0.977 to 0.999, with a mean of 0.995. The largest mean square for error was 0.1633

TABLE 4. Summary results from description of characters by Equation (3). See text and Table 1 for abbreviations. No entry in the last column indicates that *f(n)* effects were not significant at $P < 0.25$.

Variable	Model R^2	Expected means for crosses AA	AO	OA	OO	Probability level of cross effects	Probability level of *f(n)* effects
			Data from Table 2 (n = 27)				
% hatch	0.552	44	54	45	70	0.234	
Weight at 30 d	0.930	0.18	0.17	0.13	0.10	0.0001	
			Data from Table 3 (n = 36)				
A	0.546	74	65	57	51	0.0011	0.095
k	0.567	0.018	0.021	0.018	0.023	0.0004	
t_0	0.484	326	292	318	277	0.0033	
Wt125d	0.487	2.05	2.10	1.91	1.71	0.0900	
Wt250d	0.523	15	21	13	19	0.0143	
Wt325d	0.573	36	43	30	38	0.0122	
Wt13cm	0.509	22.8	21.4	21.2	20.7	0.0027	0.0625
Wt17cm	0.563	51.0	49.0	49.2	47.9	0.0122	0.0953
CV125d	0.758	20	24	26	31	0.0013	0.0471
CV325d	0.638	45	48	68	36	0.0008	0.1231

and the average 0.0949. As with other interpolated values, many values of the predicted mean weights at 13 and 17 cm (Wt13cm and Wt17cm, Table 1) are significantly different from each other given this error.

Distribution of pooled data from Survey 3 are shown in Fig. 1. In 23 of 36 tanks in Survey 3, the weight frequency distributions were not significantly different from normal (Kolmogorov D-statistic > 0.05) (Stephens 1974). In general, a CV $> 40\%$ in Table 3 indicates a positively skewed distribution, that is the weights were clustered at values less than the mean. All OO tank populations were normally distributed. Five of eight AA populations were normally distributed, two were positively skewed, and one was negatively skewed. All but two of the OA distributions were positively skewed. A variety of AO distributions occurred: one was negatively skewed and three were positively skewed.

Comparison of Characters

Hatching success, which varied between 0 and 93% (Table 2), was not related to cross type (Table 4). Another model testing only dam (d_i) and sire (s_j) (% hatch $= \mu + d_i + s_j + \epsilon$) was also fitted to nonzero results from Table 2. The overall model had an R^2 value of 0.890, with dam effects significant at the $P < 0.01$ level and sire effects at the $P \sim 0.18$ level. Dam effects were even more obvious if failed crosses were also considered.

Mean weights of fish at 30 d (Wt30d) in different families were related to cross type (Tables 2 and 4). The least squares means were ordered as follows: AA > AO > OA > OO, with AA and AO means weights different from each other at $P \sim 0.11$ and OA and OO significantly different from all others at $P < 0.05$ (t-tests using error from Equation (3) (Table 4). The trend was also similar in the five mating sets (Table 2).

The rank orders of the mean weights at 125 d (Wt125d) were AO > AA > OA > OO, again with AO and AA not significantly different at $P > 0.05$ (Table 4). Asymptotes (A), mean weights at 13 cm length (Wt13cm), and the mean weight at 30 d (Wt30d) were correlated with Wt125d, and values had the rank orders AA > AO > OA > OO. The CV at 125 d (CV125d) had the reverse rank order and was also correlated with the above characters. (Correlations between values in columns in Table 3 are not given.)

The rank order of mean weights at 325 d was AO > OO > AA > OA (Table 4), also evident in several mating sets. The growth rate (k) and the CV at 325 d (CV325d) had a similar but reversed trend (Table 4). The mean weight at 17 cm (Wt17cm) was more strongly correlated with Wt325d, k, CV325d, and Wt17cm than with those described in the previous paragraph, namely Wt125d, A, Wt13cm, and Wt30d.

The crosses with NAFs, AA and AO, had an intermediate increase (average of 22%) in relative variation (CV) between 125 and 325 d. The increase in OA families averaged 42%. The increase in OO families averaged 5% during the same growth interval. These differences are also indicated within mating sets (Table 3). In each cross type, the change in mean weights between 125 and 325 d is inversely proportional to the change in the CVs in the same interval.

The largest individual fish in the experiments were hybrids (Fig. 1; last column, Table 3). Hybrids generally had intermediate pigmentation and a larger diversity of colour patterns than their parents, but this was not quantified.

The marginal significances of the corrections for the number of fish per tank (Equation (3); Table 4) predict that the characters A, Wt13cm, Wt17cm, and CV325d were smaller in value in tanks with 200 rather than 100 fish. The effects of the number of fish are small compared with cross-type differences.

When fish from these stocks were 2 yr old, 3% of the NOMs were sexually mature. Only 0.3% of the AO and OA males were mature, and none of the NAMs matured. Even though exceptionally large fish were separated from the AO families, the remaining fish stayed slim, which suggested that they remained sexually immature. By 3 yr, when only pure stocks were maintained, most of the NOMs were mature, while the NAMs were not.

Discussion

The mean weights of subyearlings in the four crosses were related to (1) maternal effects near hatching and swim-up, (2) additive strain effects for approximately 4 mo, and then

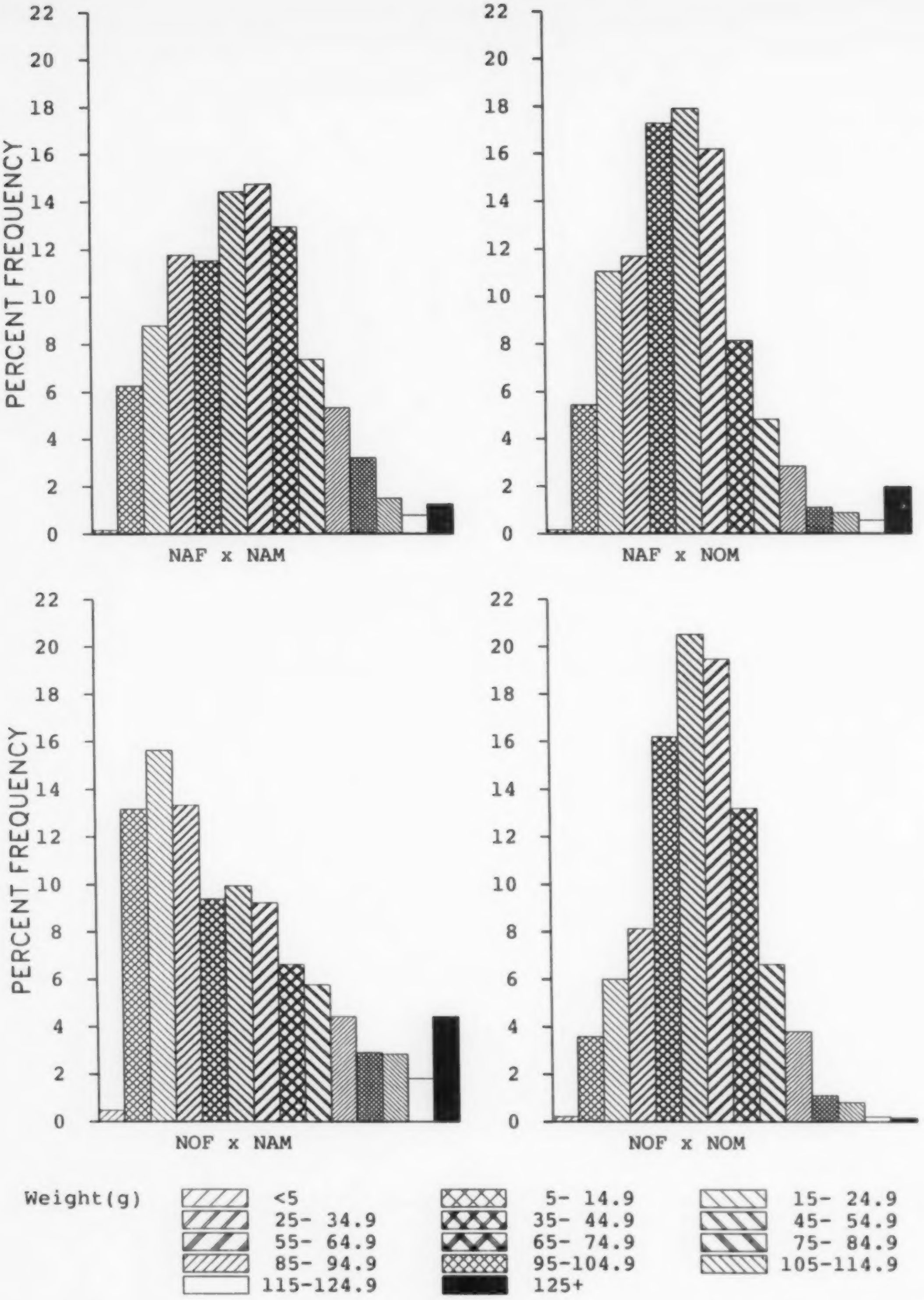

FIG. 1. Frequency of weight classes in four types of crosses with Arctic charr. Pooled data from all tanks in Survey 3, representing between 1267 and 1723 fish of each type, are presented.

(3) dominance interactions and/or heterozygosity effects until the age of 1 yr. Mean weights were also related to the number of fish per tank, but this factor accounted for a small part of the observed variability.

As in a previous study with Labrador charr (de March 1991), hatching success was entirely an individual dam effect and was not related to other experimental factors, in this case the sire or the strain.

Early growth, estimated by Wt30d and Wt125d, may have been partly related to maternal effects, but is best described as due to additive genetic effects. The early juveniles of NAFs, whether sired by NAFs or NOMs, were considerably larger than those of NOFs. This difference was perhaps related to strain differences in egg size. The offspring of NAMs, however, were larger than the offspring of NOMs in spite of the strong dam effect. The difference between the contribution of the pure strains was not unexpected, since differences between them have been previously demonstrated (Baker 1983). In a previous study with Labrador charr, additive genetic effects for growth at early life stages were demonstrated for individual parents within a strain (de March 1991); in this study, additive effects at early stages are demonstrated for hybrids of two strains. Additive effects also describe the early (125 d) patterns of CVs. The CVs of both hybrids are intermediate between those of the pure strains (Tables 3 and 4).

The simple patterns of maternal and additive genetic effects became obscured after 125 d. The fact that the OA hybrid families grew very slowly in the latter half of the experiment allows no simple explanation of the trends. Neither the final weights, the CVs, nor the changes in either character can be explained in terms of maternal effects, additive genetic effects, or hybrid vigour. Evidence for maternal effects remained: AA and AO crosses remained similar for the duration of the experiment. The existence of hybrid vigour cannot be dismissed, since the AO hybrids did demonstrate slightly superior growth in general and since individuals of both types of hybrids were the largest in the entire experiment. No evidence of additive genetic effects remained at 325 d.

Numerous environmental and genetic hypotheses can be invoked to explain such interaction effects. Similar effects have not been reported in the literature; hence, testing of hypotheses required further experimentation or knowledge of mechanisms of gene action.

Environmental causes merit consideration. At any point in time, offspring from NAFs were 2 wk older than offspring from NOFs; thus, maternal effects could have been confounded by unexpected events affecting all tanks. For example, at one point, food rations were reduced from 150 to 100% of suggested rations to reduce clogging of the biological filters. It is possible that the growth of smaller fish was affected by water quality parameters when filters were taxed. Also, it is possible that the OA juveniles experienced some feeding problems if the size distributions in the populations were positively skewed. Feed particle sizes were determined by the size of the average fish, not by the size of the most fish. However, if either of the above complications did affect results, the OO crosses should have been affected as much as the OA crosses, and this was not the case.

The simplest genetic hypothesis to explain the final results would be one involving epistatic interactions of loci on sex chromosomes. It has been suggested to me that increasing variance may be the first sign of male sexual maturation. In my experiment, increased variance occurred mainly in the OA families. It is unlikely that this was caused by precocious or early-maturing males not growing, since these were not mature by 3 yr. It is still possible that the sex chromosomes from NOMs contribute to somatic growth more than those from NAMs.

The inverse correlations between final weights (or changes in weights) and the CVs (or the change in CVs), evident here both within and among cross types, and also in a previous study with Labrador charr (de March 1991), will be examined in more detail elsewhere. The development of unpredictable size variability in Arctic charr has been of interest at the Rockwood Aquaculture Research Centre since the species has been cultured, and a number of studies have been attempted to identify the environmental causes of the variability. At this point in time, I believe the variability is a normal property of families within strains and perhaps Arctic charr in general. Possible genetic hypotheses describing such relationships still must be made and perhaps contribute to the understanding of size distributions of Arctic charr in more natural environments.

This study suggests that the culture of hybrids may be a good hatchery production strategy, but that the hybrids must be chosen carefully. The AO hybrids combine the desirable characteristics of good growth, low variation, and possibly desirable colouration. The reciprocal hybrid, on the other hand, is unsatisfactory.

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References

AYLES, G. B. 1974. Relative importance of additive genetic and maternal sources of variation in early survival of young splake hybrids (*Salvelinus fontinalis* × *S. namaychus*). J. Fish. Res. Board Can. 31: 1499–1502.

AYLES, G. B., AND R. F. BAKER. 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes and Canadian prairies. Aquaculture 33: 269–280.

BAKER, R. 1983. Effect of temperature, size and rations on the growth of two strains of Arctic charr (*Salvelinus alpinus*). M.Sc. thesis, University of Manitoba, Winnipeg, Man. 227 p.

BAKOS, J. 1979. Crossbreeding Hungarian races of common carp to develop more productive hybrids, p. 635–642. *In* T. V. R. Pillay and W. A. Dill [ed.] Advances in aquaculture. Fishing News Books Ltd., Farnham.

1987. Selective breeding and intraspecific hybridization of warm water fishes, p. 303–311. *In* K. Tiews, [ed.] Selection, hybridization, and engineering in aquaculture. Vol I. H. Heenemann GmbH & Co., Berlin.

BECKER, W. A. 1984. Manual of quantitative genetics, 4th ed. Academic Enterprises, Pullman, WA. 195 p.

CHEVASSUS, B. 1979. Hybridization in salmonids: results and perspectives. Aquaculture 17: 113–128.

DE MARCH, B. G. E. 1991. Genetic, maternal, and tank determinants of growth in hatchery-reared juvenile Arctic charr *Salvelinus alpinus*. Can. J. Zool. 69: 655–660.

GALL, G. A. E. 1969. Quantitative inheritance and environmental response of rainbow trout, p. 177–184. *In* O. W. Neuhaus and J. E. Halver [ed.] Fish in research, Academic Press, New York, NY.

KATO, T. 1966. Studies of the techniques of salmon and trout culturing. 3. Growth and survival of *Salvelinus pluvius*, *Salvelinus fontinalis fontinalis* and the hybrid *Salvelinus pluvius* × *Salvelinus fontinalis fontinalis*. Bull. Freshwater Fish. Res. Lab. Tokyo 16: 59–65.

KRAZNAI, Z. L. 1987. Intraspecific hybridization of warm water finfish, p. 35–46. *In* K. Tiews [ed.] Selection, hybridization, and engineering in aquaculture. Vol I. H. Heenemann GmbH & Co., Berlin.

LEITRITZ, E., AND R. C. LEWIS. 1980. Trout and salmon culture (hatchery methods). Calif. Fish Bull. 164: 197 p.

Moav, R. 1979. Genetic improvement in aquaculture industry, p. 610–622. *In* T. V. R. Pillay and W. A. Dill [ed.] Advances in aquaculture. Fishing News Books Ltd., Farnham.

Moav, R., G. Hulata, and G. Wohlfarth. 1974. Genetic differences between Chinese and European races of the common carp. 1. Analysis of genotype–environment interactions for growth rate. Heredity 34: 323–340.

Naevdal, G., and P. Dalpadado. 1987. Intraspecific hybridization — cold water species, p. 23–33. *In* K. Tiews [ed.] Selection, hybridization, and engineering in aquaculture. Vol I. H. Heenemann GmbH & Co., Berlin.

Papst, M. H., and G. E. Hopky. 1984. Development of an Arctic charr (*Salvelinus alpinus*) brood stock. Aquaculture 43: 323–331.

Refstie, T. 1983. Hybrids between salmonid species, growth rate and survival in seawater. Aquaculture 33: 281–285.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

SAS Institute Inc. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute Inc., Cary, NC. 956 p.

Stephens, M. A. 1974. EDF statistics for goodness of fit and some comparisons. J. Am. Stat. Assoc. 69: 730–737.

Suzuki, R. 1976. The culture of common carp in Japan, p. 161–166. *In* T. V. R. Pillay and W. A. Dill [ed.] Advances in aquaculture. Fishing News Books Ltd., Farnham.

Webster, D. A., and W. A. Flick. 1981. Performance of indigenous, exotic, and hybrid strains of brook trout (*Salvelinus alpinus*) in water of the Adirondack Mountains, New York, Can. J. Fish. Aquat. Sci. 38: 1701–1707.

Enzyme Activities of Gill, Hepatopancreas, Mantle, and Adductor Muscle of the Oyster (*Crassostrea virginica*) after Changes in Diet and Salinity

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We examined the effects of exposure to low salinity (1/3 seawater) on the maximal activities of enzymes of lipid oxidation, amino acid and ketone body metabolism, gluconeogenesis, and glycolysis in gill, mantle, hepatopancreas, and adductor muscle of the oyster (*Crassostrea virginica*). No significant metabolic reorganization occurred after 4–6 wk in low salinity. The few significant differences which were found between enzyme activities of high- and low-salinity treatment groups were always due to lower activities in the low-salinity group. In particular, hexokinase, fructose bisphosphatase, and 3-hydroxyacyl CoA dehydrogenase declined in gill, hepatopancreas, and adductor muscle. Since high ionic strength depresses the activity of many enzymes, the reduced ionic strength in the cells of the low-salinity group may perturb metabolism by elevating rates of enzyme activity. As indicated by the present study, part of the response to low salinity by euryhaline molluscs may involve reducing the activity of certain enzymes to achieve a homeostasis of metabolic function.

On a étudié les effets de la salinité réduite (1/3 d'eau salée) sur l'activité maximale des enzymes de l'oxydation lipidique, du métabolisme des acides aminés et des corps cétoniques, de la gluconéogenèse et de la glycolyse dans les branchies, le manteau, l'hépato-pancréas et le muscle adducteur de l'huître (*Crassostrea virginica*). Aucune réorganisation métabolique n'était notable au bout de 4 à 6 sem en salinité réduite. Les quelques différences significatives observées entre les groupes gardés à salinité élevée et réduite étaient toujours dues à des activités enzymatiques moindres chez le groupe gardé à salinité réduite. Plus particulièrement, on a noté la baisse de l'activité de l'hexokinase, de la fructose-biphosphatase et de la 3-hydroxyacyl-CoA-déshydrogénase dans les branchies, l'hépatopancréas et le muscle adducteur. Étant donné qu'à concentration ionique élevée l'activité de nombreuses enzymes diminue, le fait que la concentration ionique soit réduite dans les cellules des huîtres gardées à salinité réduite peut perturber le métabolisme en stimulant l'activité enzymatique. Comme on l'a constaté dans l'étude présentée ici, en partie, la réaction des mollusques euryhalins à une baisse de salinité peut être une diminution de l'activité de certaines enzymes pour le maintien de l'homéostase de la fonction métabolique.

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Changes in extra- (Hand and Stickle 1977) and intracellular inorganic ion levels (Bricteux-Gregoire et al. 1964) occur in response to changing external salinities in many osmoconforming marine invertebrates. Such changes may have substantial effects on the metabolism of these organisms. Recent investigations of the oxidative properties of mitochondria of marine bivalves indicate a stimulation of oxidation of substrates from a variety of metabolic pathways in response to an abrupt shift to low osmolarities. Oxidation of pyruvate (Ballantyne and Moyes 1987a), some but not all amino acids (Ballantyne and Storey 1983, 1985; Ballantyne and Moon 1985; Ballantyne and Moyes 1987b, 1987c; Moyes and Ballantyne 1987), and some chain lengths of acyl carnitines (Ballantyne and Storey 1984; Ballantyne and Moyes 1987a) is enhanced at low salinities.

Some of the increases in respiration found in isolated mitochondria under hypoosmotic conditions are likely due to increased enzyme activity at low ionic strength. Investigations of the effects of acute hypoosmotic stress on the oxidation of glutamate in oyster gill mitochondria indicate that changing ionic strength in the mitochondrial matrix stimulates the electron transport chain resulting in elevated oxidation (Ballantyne and Moyes 1987c). Numerous metabolic enzymes are influenced by changing ionic strength (Chaplin et al. 1965; Gilles 1969; Wood 1973; Sarkissian 1974; Sugden and Newsholme 1975). The effects of changes in intracellular ionic strength as occur during salinity acclimation in euryhaline molluscs may disrupt metabolism by affecting enzyme activity. To maintain metabolic function in the face of lower intracellular ionic strength, adjustments in the levels of some metabolic enzymes may occur with long-term acclimation to hypoosmotic stress (Gilles 1969; Ballantyne et al. 1987).

Long-term metabolic responses to changing salinity have not been investigated in pathways other than those involving amino acids. The present study was undertaken to determine the effects of exposure to low salinity on the activities of enzymes of various metabolic pathways in tissues of the oyster (*Crassostrea virginica*). In addition, to assess changes occurring in the metabolism of the oysters during the treatment period due to holding conditions or diet, the levels of enzymes were measured at the beginning and the end of the treatment period.

Materials and Methods

Animals

Malpeque oysters were obtained from a local seafood supplier and maintained at the Marine Biology Laboratory at the University of Guelph in seawater (1000 mosmol/L) for 2 wk before beginning the experiments. The animals were obtained in June and initial measurements were made within 1 wk. The other measurements were made in August after the treatment period. Each week throughout the study the oysters were fed algae and emulsions of herring. The temperature was 10 ± 1°C throughout the study. Animals were transferred directly to 1/3-strength seawater or retained in full-strength seawater for 4–6 wk before enzymes were measured. Ammonium ion levels in the water were monitored weekly and did not rise above 0.2 mg/L during the study.

Tissue Preparation

Tissues were removed from animals immediately after removal from the water and placed in ice-cold buffer (50 mM imidazole, pH 7.4, at 20°C). In the case of hydroxymethylglutaryl-CoA lyase, 3-oxoacid transferase, acetoacetyl-CoA thiolase, carnitine palmitoyl transferase, and carnitine octanoyl transferase, 0.2% triton X-100 was added to the homogenization buffer. The tissue was homogenized with a Polytron tissue homogenizer using three 10-s bursts. The homogenizer was set at a submaximal speed (five of nine possible settings). The homogenate was centrifuged at 18 200 × *g* for 20 min. The resulting supernatant was used in subsequent assays.

Enzyme Measurements

All enzyme measurements were carried out in duplicate at 10°C. Substrate concentrations and amounts of coupling enzyme were optimized with tissues from full-strength seawater animals. Hepatopancreas enzymes were always measured first and citrate synthase and carnitine octanoyl transferase and carnitine palmitoyl transferase were measured first in all tissues. NADH was prepared every 4 h and all other substrates were prepared fresh daily. Conditions for each assay are outlined below.

Hexokinase (HK) (EC[1] 2.7.1.1): 50 mM imidazole, pH 7.1, at 20°C, 1 mM glucose, 5 mM $MgCl_2$, 1 mM ATP, 0.16 mM NADP, 2 units glucose-6-phosphate dehydrogenase (G6PDH).

Pyruvate kinase (PK) (EC 2.7.1.40): 50 mM imidazole, pH 7.1, at 20°C, 5 mM ADP, 10 mM $MgCl_2$, 0.2 mM NADH, 50 mM KCl, 5.0 mM phosphoenolpyruvate, 1 unit lactate dehydrogenase (LDH).

Fructose 1,6-bisphosphatase (FBPase) (EC 3.1.3.11): 50 mM imidazole, pH 7.1, at 20°C, 15 mM $MgCl_2$, 0.1 mM FBPase, 0.2 mM NADP, 2 units G6PDH, 10 units phosphoglucose isomerase (PGI).

Glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49): 50 mM imidazole, pH 7.1, at 20°C, 7 mM $MgCl_2$, 0.4 mM NADP, 1 mM glucose-6-phosphate.

Glutamate pyruvate transaminase (GPT) (EC 2.6.1.2): 50 mM imidazole, pH 7.1, at 20°C, 200 mM L-alanine, 0.2 mM NADH, 0.025 mM pyridoxal phosphate, 1 unit LDH, 10.5 mM α-ketoglutarate.

Glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1): 50 mM imidazole, pH 7.1, at 20°C, 0.2 mM NADH, 0.25 mM pyridoxal phosphate, 30 mM aspartate, 7 mM α-ketoglutarate, 1 unit malate dehydrogenase (MDH).

Glutamate dehydrogenase (GDH) (EC 1.4.1.2): 50 mM imidazole, pH 8.1, at 20°C, 250 mM ammonium acetate, 0.1 mM EDTA, 0.1 mM NADH, 1 mM ADP, 14 mM α-ketoglutarate.

Citrate synthase (CS) (EC 4.1.3.7): 50 mM imidazole, pH 8.0, at 20°C, 0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.3 mM acetyl-CoA, 0.5 mM oxaloacetate.

Carnitine octanoyl transferase (COT) (EC 2.3.1.21): 50 mM imidazole, pH 8.1, at 20°C, 5 mM L-carnitine, 1 mM DTNB, 50 μM octanoyl-CoA.

Carnitine palmitoyl transferase (CPT) (EC 2.3.1.21): 50 mM imidazole, pH 8.1, at 20°C, 5 mM L-carnitine, 1 mM DTNB, 50 μM palmitoyl-CoA.

β-Hydroxybutyrate dehydrogenase (BHBDH) (EC 1.1.1.30): 50 mM imidazole, pH 8.1, at 20°C, 2 mM NAD, 10 mM β-hydroxybutyrate, 0.3 μg rotenone/L.

3-Hydroxyacyl-CoA dehydrogenase (HOAD) (EC 1.1.1.35): 50 mM imidazole, pH 8.1, at 20°C, 0.2 mM acetoacetyl-CoA, 0.1 mM NADH.

Acetoacetyl-CoA thiolase (AA-CoA thiolase) (EC 2.3.1.9): 50 mM imidazole, pH 7.2, at 20°C, 5 mM $MgCl_2$, 0.12 mM CoA, 0.07 mM acetoacetyl-CoA, measured at 303 nM.

3-Oxoacid transferase (OAT) (EC 2.8.3.5): 50 mM imidazole, pH 7.2, at 20°C, 5 mM iodoacetamide, 0.4 mM succinyl-CoA, 5 mM $MgCl_2$, 50 mM acetoacetate, measured at 313 nM.

Hydroxymethylglutaryl-CoA lyase (HMG-CoA lyase) (EC 4.1.3.4): 50 mM imidazole, pH 8.0, at 20°C, 10 mM $MgCl_2$, 0.2 mM NADH, 0.2 mM HMG-CoA, 1 unit BHBDH.

Statistics

Within each tissue and for each enzyme, an analysis of variance of activities was performed among control, full-strength seawater, and low-salinity treated groups using the general linear models procedure of the SAS (Statistical Analysis System) Institute Inc., Box 8000, Cary, NC 27511-8000. Mean enzyme activities were compared using Scheffe's multiple comparison technique with the probability of making a type I error set at 0.05.

Chemicals

All chemicals and biochemicals were obtained from the Sigma Chemical Co., St. Louis, MO, and were of the highest purity available.

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[1] EC refers to the system of nomenclature recommended by the Commission on Biochemical Nomenclature, 1972.

Results

Gill

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of HK ($p < 0.0001$)(FBPase ($p < 0.0018$), GDH ($p < 0.0158$), and HOAD ($p < 0.004$) (Table 1). There were significantly lower activities of HK ($p < 0.0001$), FBPase ($p < 0.0018$), and HOAD ($p < 0.004$) in the low-salinity exposed group compared with the group maintained in full-strength seawater salinity (Table 1). Compared with other tissues, OAT and G6PDH activities were highest in all treatment groups. CS was highest in gill compared with other tissues in control and full-strength seawater and low-salinity treated animals but not in control animals. The gill had the highest GDH activity compared with other tissues only in low-salinity animals.

Hepatopancreas

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of HK ($p < 0.0001$), FBPase ($p < 0.0001$), GDH ($p < 0.0005$), and HOAD ($p < 0.0001$) (Table 2). Significantly lower activities of HK ($p < 0.0001$), FBPase, and HOAD ($p < 0.0001$) were measured in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 2). The highest activities of HOAD, COT, and CPT were found in this tissue in all groups. HK and AA-CoA thiolase were highest in hepatopancreas compared with other tissues in control (full-strength seawater) and low-salinity animals. Hepatopancreas had the highest GOT and GPT but not GDH compared with other tissues in full-strength seawater animals. The highest FBPase levels in control and full-strength seawater but not low-salinity animals were observed in hepatopancreas compared with other tissues.

Mantle

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L for HK ($p < 0.0001$), FBPase ($p < 0.0001$), CS ($p < 0.0007$), and HOAD ($p < 0.0033$) (Table 3). As well, there were significantly lower activities for HK ($p < 0.0001$), FBPase ($p < 0.0001$), and CS ($p < 0.0007$) in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 3). BHBDH could not be detected in any of the tissues (activity < 0.003 U/g).

Adductor Muscle

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of PK ($p < 0.0033$) and HOAD ($p < 0.0083$) (Table 4). There were significantly lower ($p < 0.0083$) activities of HOAD in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 4). The highest HMG-CoA lyase activities were found in this tissue in full-strength seawater and low-salinity treated animals compared with other tissues. GPT, GOT, and GDH were highest in adductor muscle compared with other tissues in control and low-salinity treated animals but not in full-strength seawater animals. Adductor muscle had the highest FBPase activity in low-salinity animals compared with other tissues.

Discussion

Metabolic Organization of the Oyster

All tissues examined demonstrated HOAD, COT, and CPT activity, indicative of the ability to oxidize fatty acids. Lipid oxidation may play an important role in the metabolism of most tissues of the oyster. This contrasts with the apparent inability of the molluscan heart to oxidize lipids (Ballantyne et al. 1981; Ballantyne and Storey 1983). The levels of COT and CPT indicate fatty acid oxidation proceeds in a carnitine-dependent manner in agreement with our previous studies of acyl carnitine oxidation in mitochondria isolated from hepatopancreas of other species of bivalves (*Mercenaria mercenaria*, Ballantyne and Storey 1984; *Mytilus edulis*, Ballantyne and Moon 1985) and gill of *Crassostrea virginica* (Ballantyne and Moyes 1987a). The hepatopancreas has the highest levels of these enzymes,

TABLE 1. Activities of enzymes of intermediary metabolism in the gill of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.077 ± 0.104 (6) a	0.142 ± 0.142 (6) a	0.431 ± 0.118 (6) b
PK	1.138 ± 1.674 (6) a	0.368 ± 0.124 (6) a	0.366 ± 0.174 (6) a
FBPase	0.019 ± 0.104 (7) a	0.040 ± 0.020 (8) a	0.078 ± 0.023 (6) b
G6PDH	2.226 ± 0.553 (6) a	1.836 ± 0.344 (6) a	2.376 ± 0.339 (6) a
GPT	1.147 ± 0.430 (6) a	1.377 ± 0.279 (6) b	1.932 ± 0.506 (6) ab
GOT	6.148 ± 1.872 (6) a	4.272 ± 1.003 (6) a	4.763 ± 1.627 (6) a
GDH	0.342 ± 0.045 (6) a	0.213 ± 0.054 (6) b	0.200 ± 0.053 (6) b
CS	2.378 ± 0.868 (6) a	1.558 ± 0.765 (6) a	2.676 ± 0.743 (6) a
COT	0.020 ± 0.017 (5) a	0.015 ± 0.006 (3) a	0.037 ± 0.007 (5) a
CPT	0.015 ± 0.034 (4) a	0.034 ± 0.014 (7) a	0.041 ± 0.014 (4) a
HOAD	0.159 ± 0.033 (6) a	0.190 ± 0.043 (6) a	0.301 ± 0.087 (6) b
AA-CoA thiolase	0.081 ± 0.047 (6) a	0.116 ± 0.049 (8) a	0.060 ± 0.032 (6) a
OAT	0.028 ± 0.010 (6) a	0.015 ± 0.012 (8) a	0.015 ± 0.007 (6) a
HMG-CoA lyase	0.011 ± 0.013 (6) a	0.030 ± 0.013 (8) b	0.025 ± 0.020 (5) ab
BHBDH	<0.003	<0.003	<0.003

TABLE 2. Activities of enzymes of intermediary metabolism in the hepatopancreas of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.262 ± 0.059 (8) a	0.217 ± 0.068 (6) a	0.442 ± 0.064 (6) b
PK	1.602 ± 1.391 (6) a	0.395 ± 0.133 (6) a	0.522 ± 0.242 (6) a
FBPase	0.044 ± 0.022 (6) a	0.051 ± 0.034 (8) a	0.164 ± 0.022 (5) b
G6PDH	1.550 ± 0.332 (6) a	0.984 ± 0.164 (6) a	1.565 ± 0.689 (6) a
GPT	2.562 ± 1.473 (6) a	2.171 ± 1.552 (6) a	4.763 ± 1.769 (5) a
GOT	6.408 ± 2.021 (6) a	4.272 ± 1.003 (6) a	5.412 ± 0.834 (6) a
GDH	0.168 ± 0.037 (6) a	0.088 ± 0.019 (6) b	0.083 ± 0.029 (6) b
CS	1.995 ± 0.643 (6) a	1.775 ± 0.430 (5) a	2.127 ± 0.579 (6) a
COT	0.024 ± 0.034 (6) a	0.031 ± 0.017 (5) a	0.054 ± 0.025 (6) a
CPT	0.044 ± 0.034 (3) a	0.081 ± 0.033 (4) a	0.056 ± 0.033 (6) a
HOAD	0.194 ± 0.062 (6) a	0.241 ± 0.104 (6) a	0.597 ± 0.117 (6) b
AA-CoA thiolase	0.165 ± 0.056 (8) a	0.198 ± 0.121 (8) a	0.236 ± 0.144 (6) a
OAT	0.019 ± 0.007 (6) a	0.011 ± 0.014 (4) a	0.013 ± 0.009 (5) a
HMG-CoA lyase	0.044 ± 0.018 (6) a	0.028 ± 0.031 (4) a	0.039 ± 0.017 (6) a
BHBDH	<0.003	<0.003	<0.003

TABLE 3. Activities of enzymes of intermediary metabolism in the mantle of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.134 ± 0.090 (5) a	0.032 ± 0.018 (5) c	0.339 ± 0.048 (6) b
PK	1.748 ± 2.769 (6) a	0.304 ± 0.097 (6) a	0.276 ± 0.131 (6) a
FBPase	0.027 ± 0.022 (5) a	0.030 ± 0.009 (8) a	0.093 ± 0.012 (6) b
G6PDG	1.304 ± 0.210 (6) a	1.073 ± 0.307 (5) a	1.556 ± 0.389 (6) a
GPT	0.943 ± 0.433 (4) a	1.226 ± 0.248 (6) a	1.685 ± 0.580 (6) a
GOT	5.947 ± 1.035 (4) a	4.341 ± 1.040 (6) a	5.078 ± 0.621 (6) a
GDH	0.273 ± 0.135 (4) a	0.124 ± 0.049 (6) a	0.269 ± 0.319 (6) a
CS	1.594 ± 0.304 (6) a	0.808 ± 0.341 (6) a	1.652 ± 0.250 (6) b
COT	0.006 ± 0.000 (1) a	0.005 ± 0.003 (3) a	0.020 ± 0.011 (6) a
CPT	0.008 ± 0.003 (5) a	0.010 ± 0.006 (4) a	0.026 ± 0.012 (5) a
HOAD	0.086 ± 0.018 (6) a	0.131 ± 0.044 (6) ab	0.176 ± 0.037 (6) b
AA-CoA thiolase	0.041 ± 0.021 (6) a	0.040 ± 0.030 (7) a	0.057 ± 0.026 (6) a
OAT	0.014 ± 0.006 (6) a	0.012 ± 0.005 (7) a	0.013 ± 0.007 (6) a
HMG-CoA lyase	0.031 ± 0.012 (6) a	0.019 ± 0.012 (7) a	0.028 ± 0.021 (6) a
BHBDG	<0.003	<0.003	<0.003

suggesting a substantial reliance on lipid as a metabolic substrate. The hepatopancreas of the oyster contains large stores of lipids, especially triglycerides (Swift et al. 1980), which could be used to supply endogenous energy requirements in addition to serving as a central storage tissue.

The levels of HK indicate that all the tissues examined are capable of using exogenous glucose to some extent. Values of HK reported for oyster adductor are similar to those determined in adductor of *Mytilus edulis* (Crabtree and Newsholme 1972) and *Ostrea edulis* (Zammit and Newsholme 1976).

CS levels indicate that gill has a slightly higher aerobic capacity compared with other tissues.

Ketone bodies have several functions in intermediary metabolism which have not been investigated in marine bivalves: (1) during starvation, higher vertebrate tissues, which normally rely extensively on glucose, use ketone bodies; (2) ketone bodies are involved in cholesterol synthesis; and (3) ketone bodies may be important in the transport of lipid carbon between tissues in marine bivalves (Ballantyne et al. 1987). The absence of detectable BHBDH activity agrees with the findings of Beis et al. (1980) in a variety of invertebrates and the very low mitochondrial oxidation of β-hydroxybutyrate in oyster gill mitochondria (Ballantyne and Moyes 1987a). Acetoacetate seems to be the predominant ketone body in marine invertebrates. The presence of OAT (the first enzyme in the pathway of acetoacetate utilization) indicates that acetoacetate can be used by all the tissues examined. The activities of OAT in oyster adductor are about 100-fold lower than those found in a more active marine mollusc, *Buccinum* (Beis et al. 1980). Levels of AA-CoA thiolase were also lower than those reported for the radular retractor muscle of the common whelk (*Buccinum undatum*) by Beis et al. (1980). This is likely due to the lower assay temperature in the present study and the lower metabolic rate of sessile bivalves. Based on tissue

TABLE 4. Activities of enzymes of intermediary metabolism in the adductor of the oyster (*Crassostrea virginica*). Values given means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.167 ± 0.035 (8) a	0.161 ± 0.093 (6) a	0.224 ± 0.049 (6) a
PK	1.138 ± 0.568 (6) a	0.350 ± 0.095 (6) b	0.356 ± 0.169 (6) b
FBPase	0.038 ± 0.038 (7) a	0.109 ± 0.061 (7) b	0.072 ± 0.032 (6) ab
G6PDH	0.855 ± 0.105 (6) a	0.634 ± 0.072 (6) a	0.817 ± 0.213 (6) a
GPT	3.021 ± 0.731 (5) a	3.127 ± 1.144 (6) a	3.167 ± 1.191 (6) a
GOT	7.363 ± 1.872 (5) a	5.693 ± 1.003 (6) a	5.343 ± 1.627 (6) a
GDH	0.386 ± 0.226 (5) a	0.166 ± 0.097 (6) b	0.180 ± 0.015 (6) ab
CS	1.604 ± 0.557 (6) a	1.194 ± 0.195 (6) a	1.383 ± 0.326 (6) a
COT	0.013 ± 0.008 (5) a	0.010 ± 0.005 (3) a	0.013 ± 0.006 (4) a
CPT	0.013 ± 0.014 (6) a	0.039 ± 0.023 (3) a	0.022 ± 0.013 (4) a
HOAD	0.115 ± 0.013 (6) a	0.130 ± 0.041 (6) a	0.195 ± 0.046 (6) b
AA-CoA thiolase	0.034 ± 0.015 (6) a	0.040 ± 0.018 (8) a	0.040 ± 0.013 (6) a
OAT	0.010 ± 0.004 (6) a	0.012 ± 0.009 (7) a	0.008 ± 0.002 (5) a
HMG-CoA lyase	0.025 ± 0.009 (6) a	0.035 ± 0.016 (8) a	0.045 ± 0.023 (6) a
BHBDH	<0.003	<0.003	<0.003

HMG-CoA lyase activities, all tissues examined have the capability to synthesize cholesterol from ketone bodies.

Amino acids are important intracellular compatible osmolytes in oyster tissues (Lynch and Wood 1966) but also serve as important oxidative substrates (Ballantyne and Storey 1983, 1985; Ballantyne and Moyes 1987b, 1987c; Ballantyne et al. 1987). GDH activities are low but similar to those reported for *Mytilus edulis* tissues (Addink and Veenhof 1975). In spite of the low activities of GDH in tissues of bivalve molluscs, this enzyme is likely important in the metabolism of amino acids during salinity stress (Moyes et al. 1985). GPT activity in mantle is similar to that reported by Awapara and Campbell (1964) for the oyster mantle. GOT activities were highest of all the enzymes examined in all tissues and in adductor are similar to those reported by Zammit and Newsholme (1976) for *Ostrea edulis* adductor muscle. The high GOT activities likely reflect the mechanism for the anaerobic production of succinate from aspartate, as has been demonstrated in oyster heart (Collicutt and Hochachka 1977).

Time-Dependent Changes in Enzyme Levels

A comparison of the changes in the full-strength seawater group from the onset of the experiment to the end of the treatment period indicates increases in HOAD activity in all tissues and FBPase and HK in all tissues except adductor. Taken together, this suggests increased fatty acid oxidation and gluconeogenesis in most tissues. Increased fatty acid oxidation may have a sparing effect on carbohydrate. Increased HK levels suggest that elevated glucose uptake in most tissues may indicate normal seasonal increases in glycogen synthesis. Wild populations of marine mussels accumulate glycogen over the same time period as the present study (Thompson 1984). Some metabolic readjustment to a new diet and laboratory conditions has, therefore, occurred. The decrease in GDH in gill and hepatopancreas suggests reduced catabolism of amino acids in these tissues, perhaps due to altered diet.

Effects of Salinity Exposure

Based on the present study, no major metabolic reorganization is apparent after low-salinity exposure for 4–6 wk. This occurs in spite of the substantial changes in intracellular organic solute content known to occur as euryhaline molluscs adapt to changing salinities (see Moyes and Ballantyne 1987 for review). It is likely that transitory changes in enzyme levels are responsible for the changing intracellular solute environment and that these are essentially complete after 4 wk.

Regulation of amino acid concentrations with salinity acclimation must require transitory changes in enzyme activity to achieve new steady-state levels. The lack of effect of exposure to different salinities on GPT activity agrees with the findings of Dupaul and Webb (1974), who found that the levels of this enzyme increased in the initial hours of high-salinity acclimation and then returned to normal levels in the subsequent hours and days. While Wickes and Morgan (1976) found that GOT and GDH varied linearly with salinity in populations of oysters from different salinities, such differences may reflect genetic heterogeneity rather than acclimation responses. The highest GDH in low-salinity animals occurred in gill. Greater GDH function in this tissue in low-salinity animals identifies this tissue as the major site of deamination in low salinity. Higher rates of ammonia/ammonium excretion are correlated with losses of amino acids from tissues of bivalves in low salinity (Bartenberger and Pierce 1976). GDH is known to be activated by increasing ionic strength in crustaceans (Gilles 1974; Schoffeniels 1976), but we have found oyster GDH to be inhibited by increasing ionic strength (Ballantyne and Moyes 1987c).

The lack of effect of exposure to high and low salinities on maximal activity of PK agrees with the study of Wickes and Morgan (1976) in naturally acclimated oyster adductor muscle. Other kinetic properties of PK have been shown to change with salinity acclimation in *Mytilus edulis* (Siebenaller 1979). Such changes may be involved in maintenance of metabolic homeostasis under altered intracellular conditions.

The reduced FBPase in gill, hepatopancreas, and mantle of low-salinity treated animals may indicate reduced gluconeogenesis in these tissues due to a lower glucose or glycogen requirement at these salinities. Correspondingly lower HK in each of these tissues suggests reduced tissue uptake of glucose. Taken together, these findings suggest diminished carbohydrate requirements at low salinity. It is likely that carbohydrate is conserved during periods of adequate oxygen availability (Hochachka and Somero 1984). Ballantyne and Moon (1985) reported very low oxidation of pyruvate by mitochondria isolated from the hepatopancreas of *Mytilus edulis*, indicating that carbohydrate oxidation may normally be suppressed.

The reduced activity of HOAD in low-salinity exposed adductor muscle (30% decrease), gill (40% decrease), and hepatopancreas (50% decrease) may indicate reduced reliance on lipid as an energy source at low salinity. A rationale for this observation remains to be determined.

Exposure to high and low salinities did not affect the activity of any of the enzymes associated with ketone body metabolism. Acetoacetate oxidation by isolated oyster gill mitochondria was not affected by salinity acclimation or osmolarity (Ballantyne and Moyes 1987a). An increase in the activity of HMG-CoA lyase was observed in gill with time, but activities were not affected by exposure to high or low salinities. Bivalve molluscs can synthesize cholesterol from acetate (see Voogt 1983 for review). Cholesterol is an important component of most membranes, and it changes in distribution and abundance under various environmental conditions to maintain membrane fluidity. The sterol content of *Crassostrea* changes with reproductive state (Swift et al. 1980). While the cholesterol content of membranes changes with salinity acclimation in the gills of the guppy *Poecilia reticulata* (Daikoku et al. 1982), it is not known if salinity affects the cholesterol content of marine mollusc membranes.

While the changes in metabolism outlined above may explain some of the differences between the full-strength seawater and the low-salinity groups, another explanation must be considered. Studies of oyster gill mitochondria indicate that elevated rates of oxidation at low salinities are due to reduced matrix ionic strength (Ballantyne and Moyes 1987c). It has been argued (Ballantyne and Chamberlin 1988) that enhancement of enzyme activity could occur through dilution of the intracellular inorganic ion levels. Salinity acclimation does alter the osmotic properties of isolated oyster gill mitochondria (Ballantyne and Moyes 1987b) in a manner which would tend to counteract the increased oxidation rates at low salinities. It would appear from the present study that maintenance of normal metabolic function at low salinities may involve reduced enzyme activities. This occurred in the present study for HK and CS in mantle, HK, FBPase, and HOAD in gill and adductor, and HOAD in adductor. CS activity has been shown to be inhibited by increasing ionic strength in pig heart (Wu and Yang 1970), a crustacean and sea anemone (Sarkissian and Boatwright 1974), as well as the oyster (Sarkissian 1974). The lower intracellular ionic strength in low-salinity treated oyster tissues would increase intracellular activity, necessitating a reduction in enzyme levels to compensate for this. HOAD has been found to be influenced by ionic strength in crustaceans (Chapelle and Dandrifosse 1972). In these organisms, only unphysiologically high ionic strength was inhibitory. Studies of the effects of ionic strength on molluscan HOAD are needed.

Not all enzymes have responded in the same fashion to reduced salinity due to the variation in the responses of specific enzymes to changing ionic strength. The role of ionic strength and specific ion changes in mediating metabolic changes in response to changing salinity in osmoconforming organisms requires further investigation. Based on the present study, it appears that before the significance of metabolic changes in enzyme activities can be assessed, the sensitivity of each to changing intracellular ionic strength and specific ion levels must be determined.

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References

Adding, A. D. F., and P. R. Veenhof. 1975. Regulation of mitochondrial matrix enzymes in *Mytilus edulis*. Proc. Eur. Mar. Biol. Symp. 9: 109–119.

Awapara, J., and J. W. Campbell. 1964. Utilization of $^{14}CO_2$ for the formation of some amino acids in three invertebrates. Comp. Biochem. Physiol. 11: 231–235.

Ballantyne, J. S., and M. E. Chamberlin. 1988. Adaptation and evolution of mitochondria: osmotic and ionic considerations. Can. J. Zool. 66: 1028–1035.

Ballantyne, J. S., P. W. Hochachka, and T. P. Mommsen. 1981. Studies on the metabolism of the migratory squid, *Loligo opalescens*: enzymes of tissues and heart mitochondria. Mar. Biol. Lett. 2: 75–85.

Ballantyne, J. S., and T. W. Moon. 1985. Hepatopancreas mitochondria from *Mytilus edulis*: substrate preference and effects of pH and osmolarity. Mar. Biol. 87: 239–244.

Ballantyne, J. S., and C. D. Moyes. 1987a. Osmotic effects on fatty acid, pyruvate, and ketone body oxidation in oyster gill mitochondria. Physiol. Zool. 60: 713–721.

1987b. The effects of salinity acclimation on the osmotic properties of mitochondria from the gill of *Crassostrea virginica*. J. Exp. Biol. 133: 449–456.

1987c. The role of divalent cations and ionic strength in the osmotic sensitivity of glutamate oxidation in oyster gill mitochondria. J. Exp. Biol. 130: 203–217.

Ballantyne, J. S., C. D. Moyes, and T. W. Moon. 1987. Compatible and counteracting solutes and the evolution of ion and osmoregulation in fishes. Can. J. Zool. 65: 1883–1888.

Ballantyne, J. S., and K. B. Storey. 1983. Mitochondria from the ventricle of the marine clam, *Mercenaria mercenaria*: substrate preferences and effects of pH and salt concentration on proline oxidation. Comp. Biochem. Physiol. 76B: 133–138.

1984. Mitochondria from the hepatopancreas of the marine clam, *Mercenaria mercenaria*: substrate preferences and salt and pH effects on the oxidation of palmitoyl-L-carnitine and succinate. J. Exp. Zool. 230: 165–174.

1985. Solute effects on mitochondrial respiration: the kinetics of proline oxidation by mitochondria from the ventricle of the marine clam, *Mercenaria mercenaria*. Comp. Biochem. Physiol. 81B: 777–780.

Bartenberger, C. A., and K. S. Pierce. 1976. The relationship between ammonia excretion rates and hemolymph nitrogen compounds of a euryhaline bivalve during low salinity acclimation. Biol. Bull. 150: 1–14.

Beis, A., V. A. Zammit, and E. A. Newsholme. 1980. Activities of 3-hydroxybutyrate dehydrogenase, 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase in relation to ketone-body utilisation in muscles of vertebrates and invertebrates. Eur. J. Biochem. 104: 209–215.

Bricteux-Gregoire, S., G. Duchateau-Bosson, C. Jeuniaux, and M. Florkin. 1964. Constituants osmotiquement actifs des muscles adducteurs d'*Ostrea edulis* adaptée à l'eau de mer ou à l'eau saumâtre. Arch. Int. Physiol. Biochim. 72: 267–275.

Chapelle, S., and G. Dandrifosse. 1972. Effets de différents sels sur l'activité de la deshydrogenase du β-hydroxyacyl-CoA et de la thiolase du β-cetoacyl-CoA du muscle d'*Eriocheir sinensis* et de *Homarus vulgaris*. Arch. Int. Physiol. Biochim. 80: 213–227.

Chaplin, A. E., A. K. Huggins, and K. A. Munday. 1965. Ionic effects on glutamate dehydrogenase activity from beef liver lobster muscle and crab muscle. Comp. Biochem. Physiol. 16: 49–62.

Collicutt, J. M., and P. W. Hochachka. 1977. The anaerobic oyster heart: coupling of glucose and aspartate fermentation. J. Comp. Physiol. 115: 147–157.

Crabtree, B., and E. A. Newsholme. 1972. The activities of phosphorylase, hexokinase, phosphofructokinase lactate dehydrogenase and the glycerol 3-phosphate dehydrogenase in muscles from vertebrates and invertebrates. Biochem. J. 126: 49–58.

Daikoku, T. T., I. Yano, and M. Masui. 1982. Lipid and fatty acid compositions and their changes in the different organs and tissues of guppy, *Poecilia reticulata* on sea water adaptation. Comp. Biochem. Physiol. 73A: 167–174.

Dupaul, W. D., and K. L. Webb. 1974. Salinity-induced changes in the alanine and aspartic aminotransferase activity in three marine bivalve molluscs. Arch. Int. Physiol. Biochim. 82: 817–822.

Gilles, R. 1969. Effect of various salts on the activity of enzymes implicated in amino-acid metabolism. Arch. Int. Physiol. Biochim. 77: 441–464.

1974. Studies on the effects of NaCl on the activity of *Eriocheir sinensis* glutamate dehydrogenase. Int. J. Biochem. 5: 623–628.

Hand, S. C., and W. B. Stickle. 1977. Effects of tidal fluctuations of salinity on pericardial fluid composition of the American oyster *Crassostrea virginica*. Mar. Biol. 42: 259–271.

Hochachka, P. W., and G. N. Somero. 1984. Biochemical adaptation. Princeton University Press, Princeton, NJ. 537 p.

Lynch, M. P., and L. Wood. 1966. Effects of environmental salinity on free amino acids of *Crassostrea virginica* Gmelin. Comp. Biochem. Physiol. 19: 783–790.

Moyes, C. D., and J. S. Ballantyne. 1987. Amino acid transport in bivalve mollusc mitochondria: the role of the proton motive force and monovalent cations in proline oxidation by oyster (*Crassostrea virginica*) gill mitochondria. J. Exp. Zool. 243: 1–8.

Moyes, C. D., T. W. Moon, and J. S. Ballantyne. 1985. Glutamate catabolism in mitochondria from *Mya arenaria* mantle: effects of pH on the role of glutamate dehydrogenase. J. Exp. Zool. 236: 293–301.

Sarkissian, I. V. 1974. Regulation by salt of activity of citrate synthases from osmoregulators and osmoconformers. Trans. N.Y. Acad. Sci. II, 36: 775–782.

Sarkissian, I. V., and D. T. Boatwright. 1974. Regulation by salt and by Krebs cycle metabolites of citrate from an osmoregulator, white shrimp, *Penaeus setiferus* and from a non-osmoregulator, sea anemone, *Bunedosoma cavernata*. Comp. Biochem. Physiol. 49B: 325–333.

Schoffeniels, E. 1976. Adaptations with respect to salinity. Biochem. Soc. Symp. 41: 179–204.

Siebenaller, J. F. 1979. Regulation of pyruvate kinase in *Mytilus edulis* by phosphorylation–dephosphorylation. Mar. Biol. Lett. 1: 105–110.

Sugden, P. H., and E. A. Newsholme. 1975. The effect of ammonium, inorganic phosphate and potassium ions on the activity of phosphofructokinase from muscle and nervous tissues of vertebrates and invertebrates. Biochem. J. 150: 113–122.

Swift, M. L., D. White, and M. B. Ghassemieh. 1980. Distribution of neutral lipids in the tissues of the oyster *Crassostrea virginica*. Lipids 15: 129–132.

Thompson, R. J. 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a subarctic, non-estuarine environment. Mar. Biol. 79: 277–288.

Voogt, P. A. 1983. Lipids: their distribution and metabolism, p. 329–370. *In* P. W. Hochachka [ed.] The Mollusca. Vol. 1. Metabolic biochemistry and molecular biomechanics. Academic Press Inc., New York, NY.

Wickes, M. A., and R. P. Morgan. 1976. Effects of salinity on three enzymes involved in amino acid metabolism from the American oyster, *Crassostrea virginica*. Comp. Biochem. Physiol. 53B: 339–343.

Wood, J. M. 1973. Effect of ionic strength on the activity of carnitine palmitoyltransferase I. Biochemistry 12: 5268–5273.

Wu, J., and J. T. Yang. 1970. Effects of salts and organic solvents on the activity of citrate synthase. J. Biol. Chem. 245: 3561–3564.

Zammit, V. A., and E. A. Newsholme. 1976. The maximum activities of hexokinase, phosphorylase, phosphofructokinase, glycerol phosphate dehydrogenases, lactate dehydrogenase, octopine dehydrogenase, phosphoenol pyruvate carboxykinase, nucleoside diphosphate kinase, glutamate-oxaloacetate transaminase and arginine kinase in relation to carbohydrate utilization in muscles from marine invertebrates. Biochem. J. 160: 447–462.

Intra- and Interspecific Differences in the Response of Two Lentic Species of Leeches to Seasonal Hyperoxia

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Davies, R. W., and T. E. Gates. 1991. Intra- and interspecific differences in the response of two lentic species of leeches to seasonal hyperoxia. Can. J. Fish. Aquat. Sci. 48: 1124–1127.

Intra- and interspecific differences in survivorship of *Nephelopsis obscura* and *Erpobdella punctata* exposed to hyperoxia (200 or 300%) at spring (5°C) and summer (20°C) water temperatures were examined in a flow-through system. In general, percent survival and time of 50% survival increased with leech size and decreased with higher temperature for both species. All size classes could survive the maximum recorded duration of hyperoxia in the spring, and we conclude that spring hyperoxia probably does not directly affect the ecological success or microhabitat distribution of either species. At 20°C, medium and large *E. punctata* had much higher survivorship than *N. obscura*. As summer hyperoxia can last for 30 d, these differences could contribute to changes in numerical dominance. Intra- and interspecific differences in response to hyperoxia may also influence seasonal movements between different microhabitats of different size classes of *N. obscura* and *E. punctata*. We suggest that seasonal and annual variability in littoral hyperoxia regimes can differentially influence the survivorship, reproductive success, and microhabitat distribution of many macroinvertebrates.

On a examiné les différences interspécifiques et intraspécifiques du taux de survie de *Nephelopsis obscura* et de *Erpobdella punctata* exposées à des conditions d'hyperoxie (200 ou 300 %) des eaux de printemps (5°C) et d'été (20°C) en système à débit continu. En général, le pourcentage de survie et le temps de survie de 50 % des individus ont augmenté en fonction de la taille des sangsues et ont diminué en fonction d'une augmentation de la température. Toutes les classes de tailles pouvaient survivre à la période maximum enregistrée d'hyperoxie printanière. On a donc formulé la conclusion que l'hyperoxie printanière n'influe pas directement sur le succès dans le milieu ou la distribution des microhabitats des deux espèces. À 20°C, les individus de grande taille et de taille intermédiaire de *E. punctata* ont montré un taux de survie supérieur à celui de *N. obscura*. Étant donné que l'hyperoxie estivale peut durer 30 jours, ces différences peuvent jouer un rôle dans la dominance numérique. Les différences intraspécifiques et interspécifiques de la réaction à l'hyperoxie peuvent aussi influer sur les migrations saisonnières entre les divers microhabitats dans le cas des différentes classes de tailles de *N. obscura* et de *E. punctata*. On formule l'hypothèse que la variabilité annuelle et saisonnière des régimes d'hyperoxie dans les eaux littorales peut influer par action différentielle sur le taux de survie, le succès de la reproduction et la distribution des microhabitats de nombreux macroinvertébrés.

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While it is well known that dissolved oxygen concentrations in temperate lakes vary from anoxic (or hypoxic) to hyperoxic (Hutchinson 1957) on a seasonal and sometimes diel basis, their effects on the ecology of freshwater invertebrates have been rarely examined. Physical and biological processes contribute to the heterogenous distributions of aquatic organisms, and where gradients of environmental stress exist, patterns of distribution related to differences in physiological tolerance result. The significance of anoxia to the ecology of lentic invertebrates has been examined by Nagell (1977a, 1977b, 1977c) and Nagell and Fageström (1978) working primarily with the mayfly *Cloeon dipterum* and by Davies et al. (1987) on two predatory erpobdellid leeches, *Nephelopsis obscura* and *Erpobdella punctata*. The occurrence of hyperoxia in lotic ecosystems and the effects on lotic and marine fishes have received considerable attention since the beginning of the century (Marsh and Gorham 1905) and more recently (e.g. Krise and Meade 1988; review by Weitkamp and Katz 1980). Similar marine studies have been carried out on lobsters (Hughes 1968), Mollusca (Malouf et al. 1972; Goldberg 1978; Elston 1983), shrimp (Evans and Walder 1969; Lightner et al. 1974), and crabs (Johnson 1976). The effects of hyperoxia on lotic invertebrates such as crayfish, stoneflies, mayflies, and caddisflies have been examined (Nebeker 1976; Nebeker et al. 1981). Lentic invertebrates have almost been ignored, although Nebeker (1976) and Nebeker et al. (1981) did include studies on cultured lentic *Daphnia*, midge, and mosquito larvae. Apart from a study on the histopathology, morphology, and superoxide dismutase activity of the freshwater leech *N. obscura* (Singhal and Davies 1987; Singhal et al. 1988, 1989, 1990), the effects of hyperoxia on lentic invertebrates have apparently been disregarded.

The purpose of this study was to investigate the effects of hyperoxia at summer and spring water temperatures on the survival of different size classes of *N. obscura* and *E. punctata* and to relate differences to the population dynamics and microhabitat distributions of these two species recorded in the field (Davies et al. 1977, 1981, 1982; Gates and Davies 1987; Wrona et al. 1987; Linton et al. 1983a, 1983b).

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Methods

Nephelopsis obscura and *E. punctata* were collected from Stephenson's Pond located 4.5 km northwest of Calgary, Alberta. Stephenson's Pond is a small permanent pothole lake described in detail in Davies et al. (1987) and Baird et al. (1987).

Four size classes of *N. obscura* (hatchling (5–15 mg), small (20–50 mg), medium (75–130 mg), and large (150–300 mg)) and three size classes of *E. punctata* (small (20–50 mg), medium (75–130 mg), and large (150–300 mg)) were collected from the field in late May and early June and acclimated in normoxic (100% saturated) water at either 5 or 20°C for 4 wk. All leeches were fed ad libitum for 4 h each week on *Tubifex tubifex* during the acclimation and experimental periods.

An insulated flow-through apparatus with manifolds having five experimental chambers receiving hyperoxic (200% (±1.5%) or 300% (±0.75%) saturation) water and five control chambers receiving normoxic water was used and run at 5°C (±1°C) and 20°C (±1°C) with a 12 h light : 12 h dark light regime. Each chamber consisted of a glass tube (of variable dimensions depending on the size of the leeches used) with rubber serum stopper ends. One end was pushed over a manifold outlet and two 18-gauge needles provided outflow from the other stopper.

Hyperoxic water was obtained by regulating oxygen supply to a mixing column filled with glass distillation beads. The oxygen concentrations in the experimental and control chambers were each monitored continuously by a Strathkelvin 781B oxygen meter coupled to a Radiometer E5046-0 electrode with flow cell. Water temperature was regulated by a constant-temperature bath.

For each size class of each species, 10 individuals were placed in each of the five experimental and control chambers and survivorship determined daily for 50 d. All leeches were fed ad libitum for 4 h each week on *T. tubifex*. Prey were added to and removed from the chambers with an interruption of flow not exceeding 5 s. Low field densities of lentic *E. punctata* only allowed testing at 300% saturation. Each day the activity and the incidence of the clinical signs of oxygen toxicity, i.e. depigmentation, lesion, swellings, loss of ability to attach to the substrate with the posterior sucker, as recorded by Singhal and Davies (1987), were assessed visually. Mortality was confirmed by placing apparently dead leeches in normoxic water at the experimental temperature for 24 h to see if recovery occurred.

Results

Mortality in the controls (normoxia) was extremely low and never exceeded 4%. Activity was infrequent and ventilation was never observed in control or experimental groups. As exposure time and saturation increased, the incidence of clinical signs of oxygen toxicity described by Singhal and Davies (1987) occurred in both species.

Generally, survival increased with leech size and decreased with increased temperature (Table 1; Fig. 1). Hatchling and small size classes of *N. obscura* showed lower survival in 300% than in 200% saturation at both temperatures, but the medium and large size classes only showed decreased survival with increased oxygen saturation at 20°C. The mean times for 50% survival (Stm) were 6.2 and 7.8 d for hatchling and small *N. obscura*, respectively, and 4.5 d for small *E. punctata* in 300% saturation at 20°C. The most important interspecific differences were with large *N. obscura* which had a Stm of 25.0 d in 300% saturation at 20°C compared with >50 d for large *E. punctata* under the same conditions and a Stm of 9.5 d for medium *N. obscura* compared with 15.0 d for *E. punctata*.

TABLE 1. Mean time (in days) for 50% survival (Stm) of different size classes ($n = 50$) of *N. obscura* and *E. punctata* exposed to 200 or 300% hyperoxia at 5 and 20°C.

Temperature (°C)	% saturation	Hatchling	Small	Medium	Large
N. obscura					
5	200	27.0	48.8	>50.0	>50.0
	300	11.6	19.3	>50.0	>50.0
20	200	16.3	30.5	>50.0	44.4
	300	6.2	7.8	9.5	25.0
E. punctata					
5	300	—	43.3	>50.0	>50.0
20	300	—	4.5	15.0	>50.0

Discussion

In southern Alberta, oxygen regimes in shallow prairie pothole lakes show pronounced variation on both a diel and seasonal basis. During winter under ice, hypolimnetic oxygen depletion occurs with anoxia near the sediment–water interface and severe hypoxia in the overlying water column (Baird et al. 1987). As spring approaches, the water temperature rises to about 5°C, ice and snow cover melts, and under-ice primary productivity and rapid melting of ice at the shore contribute to reoxygenation and hyperoxia (150–200%) in surface waters (Baird et al. 1987) which rarely persists for more than 1–2 d in Stephenson's Pond. During the summer (water temperatures about 20°C), hyperoxia (200–300%) persists for 1–30 d depending on wind conditions. Hyperoxia is a common phenomenon in pothole lakes and frequently causes fish mortality (Barica and Mathias 1985).

Because it produced very low or undetectable levels of superoxide dismutase (SOD), Singhal et al. (1990) concluded that *N. obscura* had two possible defenses against hyperoxia. These were to move away from areas of hyperoxia or, as also recorded by Singhal and Davies (1987), to secrete large amounts of mucus over the epidermis which reduces oxygen diffusion into the body and thereby the effects of oxygen toxicity.

In the spring when hyperoxia occurs at temperatures near 5°C the survival times for all size classes of *N. obscura* and *E. punctata* greatly exceed the maximum recorded duration of hyperoxia in the field. Thus, even though intra- and interspecific differences in survivorship occur, they are unlikely to play a direct role in the population dynamics and microhabitat distribution of these species. In contrast, intra- and interspecific differences in survivorship at summer water temperatures (20°C) are likely to play a direct role in determining population densities and microhabitat distribution of *N. obscura* and *E. punctata*. *Nephelopsis obscura* is almost always the numerically dominant lentic erpobdellid with *E. punctata* subdominant; however, occasionally the reverse situation can occur for a few years. Although morphologically and physiologically similar (Reynoldson and Davies 1980; Linton et al. 1982, 1983a, 1983b; Wrona and Davies 1984), *N. obscura* and *E. punctata* differ markedly in various aspects of their ecology

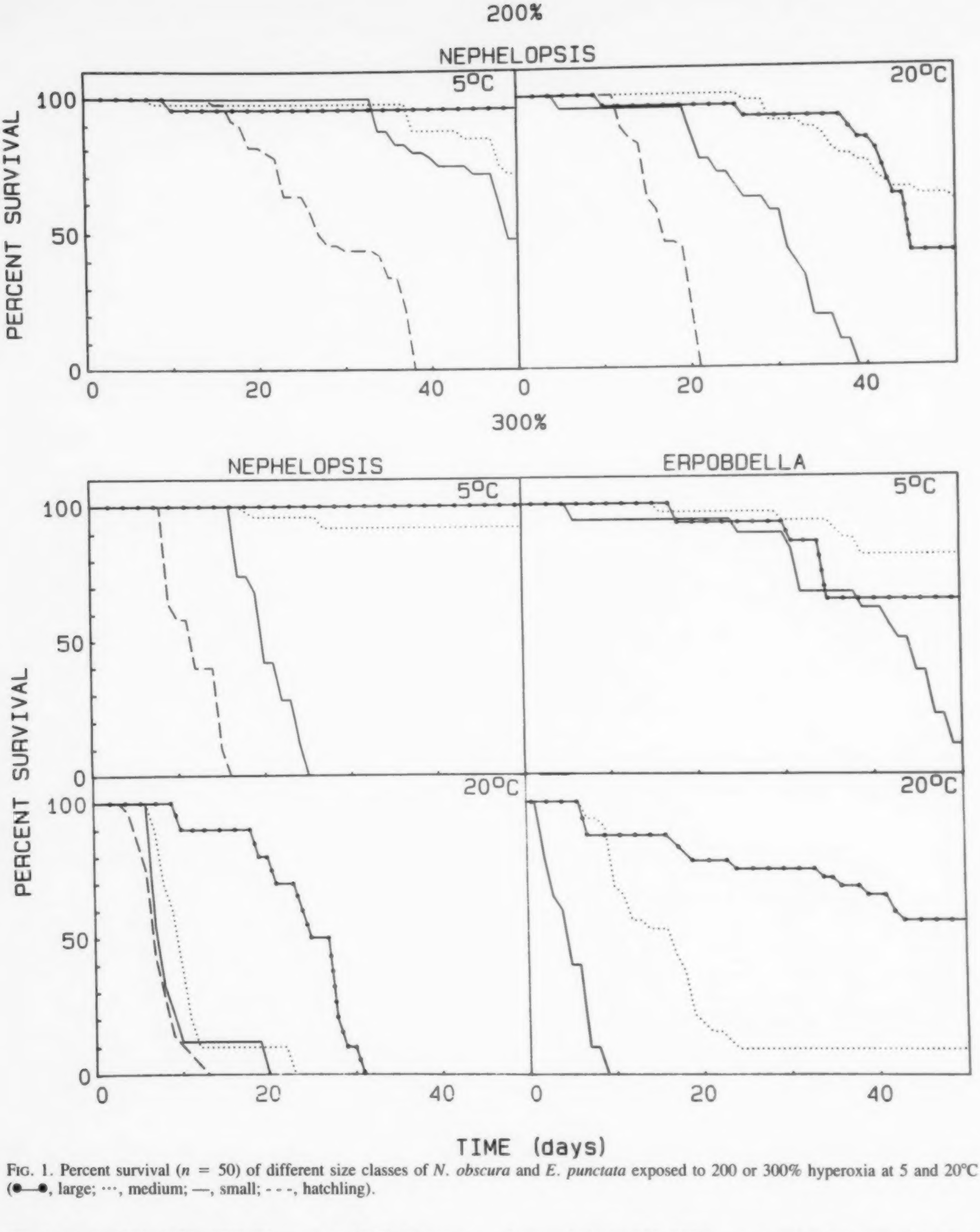

FIG. 1. Percent survival (n = 50) of different size classes of *N. obscura* and *E. punctata* exposed to 200 or 300% hyperoxia at 5 and 20°C (●—●, large; ···, medium; —, small; - - -, hatchling).

(Davies et al. 1981, 1982, 1987; Wrona et al. 1987). The occasional, short-term (2–3 yr) dominance of *E. punctata* (Davies et al. 1977, 1982) has never been explained. If during summer, hyperoxia in the 200–300% range persists for longer than 20 d, medium and large *N. obscura* would show higher mortality than comparably sized *E. punctata*, leading to increased reproductive success for the latter species. Even if *N. obscura* can detect and move out of microhabitats with hyperoxia, this would still result in reduced ecological fitness because microhabitats containing macrophytes and neighbouring rocky zones are obligate sites for cocoon deposition and successful embryo development. Thus, the relatively rare occurrence of long-term (>20 d) hyperoxia during the summer could result in higher ecological fitness for *E. punctata* and its short-term numerical dominance (Davies et al. 1977, 1982).

Davies and Everett (1977) and Davies et al. (1977) recorded intra- and interspecific differences in the timing of seasonal movements of *N. obscura* and *E. punctata* between lentic microhabitats. Temperature was studied as a stimulus for this behaviour by Gates and Davies (1986) who concluded that responses to temperature influenced movements between the shallow- and deepwater zones but could not account for movements of all size classes of both species. Davies and Everett (1977) recorded that the autumn movement to deeper water by large *N. obscura* occurred after that of smaller individuals. We have shown that small *N. obscura* are highly intolerant of hyperoxia, especially at higher temperatures, and suggest that their earlier movement to deeper water in the autumn is related to avoidance of potentially lethal oxygen conditions. Similarly, the presence of only large leeches in the macrophyte zone, where hyperoxia is more common and more persistent, is compatible with the greater tolerance of the large size classes to hyperoxic conditions.

Seasonal and annual variability in littoral hyperoxia regimes differentially influences survivorship of *N. obscura* and *E. punctata* and potentially contributes to differences in reproductive success and microhabitat distribution. Although the effects of hyperoxia on lentic macroinvertebrates in the field have been ignored, it is clear that annual and seasonal differences in hyperoxia can result directly in differential survivorship and indirectly in changes in distribution and abundance.

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References

BAIRD, D. J., T. E. GATES, AND R. W. DAVIES. 1987. Oxygen conditions in two prairie pothole lakes during winter ice cover. Can. J. Fish. Aquat. Sci. 44: 1092–1095.

BARICA, J., AND J. MATHIAS. 1985. Gas supersaturation as a cause of early spring mortality of stocked trout. Can. J. Fish. Aquat. Sci. 42: 268–279.

DAVIES, R. W., AND R. P. EVERETT. 1977. The life history, growth and age structure of *Nephelopsis obscura* Verrill 1872 (Hirudinoidea) in Alberta. Can. J. Zool. 55: 620–627.

DAVIES, R. W., T. B. REYNOLDSON, AND R. P. EVERETT. 1977. Reproductive strategies of *Erpobdella punctata* (Hirudinoidea) in two temporary ponds. Oikos 29: 313–319.

DAVIES, R. W., F. J. WRONA, AND L. R. LINTON. 1982. Changes in numerical dominance and its effects on prey utilization and interspecific competition between *Erpobdella punctata* and *Nephelopsis obscura* (Hirudinoidea): an assessment. Oikos 39: 92–99.

DAVIES, R. W., F. J. WRONA, L. R. LINTON, AND J. WILKIALIS. 1981. Inter- and intra-specific analyses of the food niches of two sympatric species of Erpobdellidae (Hirudinoidea) in Alberta, Canada. Oikos 37: 105–111.

DAVIES, R. W., T. YANG, AND F. J. WRONA. 1987. Inter- and intra-specific differences in the effects of anoxia on erpobdellid leeches using static and flow-through systems. Holarct. Ecol. 10: 149–153.

ELSTON, R. 1983. Histopathology of oxygen intoxication in juvenile red abalone, *Haliotis refescens* Swainson. J. Fish Dis. 6: 101–110.

EVANS, A., AND D. N. WALDER. 1969. Significance of gas micronuclei in the aetiology of decompression sickness. Nature (Lond.) 222: 251–252.

GATES, T. E., AND R. W. DAVIES. 1987. The influence of temperature on the depth distribution of sympatric Erpobdellidae (Hirudinoidea). Can. J. Zool. 65: 1243–1246.

GOLDBERG, R. 1978. Some effects of gas-supersaturated seawater on *Spisula solidissima* and *Argopecten irradians*. Aquaculture 4: 281–287.

HUGHES, J. T. 1968. Grow your own lobsters commercially. Ocean Ind. 3: 46–49.

HUTCHINSON, G. E. 1957. A treatise on Limnology. Vol. 1. Geography, physics, and chemistry. John Wiley & Sons, Inc., New York, NY.

JOHNSON, P. T. 1976. Gas-bubble disease in the blue crab, *Callinectes sapidus*. J. Invert. Pathol. 27: 247–253.

KRISE, W. F., AND J. W. MEADE. 1988. Effects of low-level gas supersaturation on lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 45: 666–674.

LIGHTNER, D. V., B. R. SALSER, AND R. S. WHEELER. 1974. Gas-bubble disease in the brown shrimp (*Penaeus aztecus*). Aquaculture 4: 81–84.

LINTON, L. R., R. N. DAVIES, AND F. J. WRONA. 1982. Osmotic and respirometric responses of two species of Hirundinoidea to changes in water chemistry. Comp. Biochem. Physiol. 41A: 243–247.

1983a. The effects of water temperature, ionic content and total dissolved solids on *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea, Erpobdellidae). Holarct. Ecol. 6: 59–63.

1983b. The effect of water temperature, ionic content and total dissolved solids on *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea, Erpobdellidae). Holarct. Ecol. 6: 64–68.

MALOUF, R., R. KECK, D. MAURER, AND C. EPIFANIO. 1972. Occurrence of gas-bubble disease in three species of bivalve molluscs. J. Fish. Res. Board Can. 29: 588–589.

MARSH, H. G., AND F. P. GORHAM. 1905. The gas bubble disease in fishes. Rep. U.S. Bur. Fish. 1904: 343–376.

NAGELL, B. 1977a. Survival of *Cloeon dipterum* (Ephemeroptera) larvae under anoxic conditions in water. Oikos 29: 161–165.

1977b. Overwintering of larvae of *Cloeon dipterum* (L.) in an ice covered and anoxic pond. Proc. 2nd Int. Conf. Ephemeroptera, Krakow 193–198.

1977c. Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. Oikos 29: 342–347.

NAGELL, B., AND T. FAGERSTRÖM. 1978. Adaptations and resistance of anoxia in *Cloeon dipterum* (Ephemeroptera) and *Nemoura cinerea* (Plecoptera). Oikos 30: 95–99.

NEBEKER, A. V. 1976. Survival of *Daphnia*, crayfish, and stoneflies in air-supersaturated water. J. Fish. Res. Board Can. 33: 1208–1212.

NEBEKER, A. V., F. D. BAKER, AND S. L. WEITZ. 1981. Survival and adult emergence of aquatic insects in air-supersaturated water. J. Freshwater Ecol. 1: 243–250.

REYNOLDSON, T. B., AND R. W. DAVIES. 1980. A comparative study of weight regulation in *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea). Comp. Biochem. Physiol. 66: 711–714.

SINGHAL, R. N., AND R. W. DAVIES. 1987. Histopathology of hyperoxia in *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). J. Invertebr. Pathol. 50: 33–39.

SINGHAL, R. N., R. W. DAVIES, AND M. KAPOOR. 1990. Undetectable levels of superoxide dismutase activity in the leech *Nephelopsis obscura* under hyperoxia. Arch. Hydrobiol. 119: 351–358.

SINGHAL, R. N., H. B. SARNAT, AND R. W. DAVIES. 1988. Effect of anoxia and hyperoxia on the neurons in the leech *Nephelopsis obscura* (Erpobdellidae): ultrastructural studies. J. Invertebr. Pathol. 52: 409–418.

1989. Effect of anoxia, hyperoxia and salinity on neurons in the leech *Nephelopsis obscura* (Erpobdellidae): RNA redistribution by fluorescence histochemistry. J. Invertebr. Pathol. 53: 93–101.

WEITKAMP, D. E., AND M. KATZ. 1980. A review of dissolved gas supersaturation literature. Trans. Am. Fish. Soc. 109: 659–702.

WRONA, F. J., AND R. W. DAVIES. 1984. An improved flow-through respirometer for aquatic macroinvertebrate bioenergetic research. Can. J. Fish. Aquat. Sci. 41: 380–385.

WRONA, F. J., L. R. LINTON, AND R. W. DAVIES. 1987. Reproductive success and growth of two species of Erpobdellidae: the effect of water temperature. Can. J. Zool. 65: 1253–1256.

LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

Statistical Properties of Two-Phase Surveys: Comment

Jolly and Hampton (1990) discuss the difference between model-based and design-based estimation and (quite rightly) "suggest strongly" that model-based estimators should be tested for bias and efficiency against "the appropriate design-based estimator" (p. 1283, para. 6). It is a pity that they did not feel the need to test the estimator they subsequently present (based on a two-phase strategy) against that from a conventional stratified random survey, or even to acknowledge that the two-phase strategy will introduce bias.

Jolly and Hampton's two-phase strategy is essentially the same as that I devised for trawl surveys (Francis 1984). Their equations (2) and (3) (to estimate mean fish density and its variance) are precisely equivalent to my equations (1) and (2) to estimate biomass and its variance). Where there is a slight difference is in the procedures for allocating phase 2 transects (or stations) to strata.

My phase 2 allocation procedures are iterative. There is one iteration for each phase 2 station; each iteration calculates where best to allocate the next station. The reason for this relatively complicated approach is that the alternative — Jolly and Hampton's procedure — leads to difficulties. Phase 2 allocations calculated by Jolly and Hampton's method are usually not whole numbers and, worse, can be negative (i.e. the calculated total number of transects for a stratum can be less than the number already allocated in phase 1 — see example A in Table 1). The authors do not explain how to deal with this. When none of the allocations are negative, then Jolly and Hampton's allocations, after appropriate rounding, will usually be the same as mine. However, example B in Table 1 shows that slight differences in allocation can occur. A simple calculation shows that Jolly and Hampton's allocation (after rounding) is, in this case, suboptimal.

Thus, except for the possible practical problems with Jolly and Hampton's phase 2 allocation procedures, their two-phase strategy is essentially the same as mine, and so can be expected to show similar statistical properties, viz, an increase in precision, together with a slight negative bias.

The extent of bias (and the increase in precision) to be expected from two-phase acoustic surveys has yet to be determined. The methodology of Francis (1984) provides a means to do this.

Jolly and Hampton say (in their final paragraph) that the two-phase approach may be "inappropriate and wasteful" where there is a "substantial movement of fish . . . during the course of the survey." The effect on estimated biomass (or fish density) of fish movements during a survey will depend closely on the interrelationship between these movements and the vessel track. The vessel track is perhaps more complex for two-phase than for conventional surveys, but I see no reason to assume, a priori, that fish movement will affect the estimates from one type of survey more than those from the other.

TABLE 1. Two simple examples illustrating differences between the phase 2 allocation methods of Jolly and Hampton (1990) and Francis (1984). In each case there are only two strata ($S-1$ and $S-2$) and these have areas 180 and 100, respectively; 24 transects are allocated in phase 1 and 8 in phase 2. Example A shows how Jolly and Hampton's procedure can produce a negative phase 2 allocation; example B shows that rounding Jolly and Hampton's allocation to the nearest integer does not necessarily produce the optimal result. (In these examples, I have used Jolly and Hampton's equation (9) and my "variance allocation." Similar results could be obtained using my "mean-squared allocation" and Jolly and Hampton's equation (11), as modified by the comments in the third paragraph of their Discussion.)

	Phase 1 allocation		Phase 1 SD of fish density		Phase 2 allocation			
					Francis		Jolly and Hampton	
Example	S − 1	S − 2	S − 1	S − 2	S − 1	S − 2	S − 1	S − 2
A	16	8	3.51	1.67	8	0	9.31	−1.31
B	20	4	3.95	1.16	7	1	7.51	0.49

This comment is not meant to imply any criticism of the survey approach advocated by Jolly and Hampton. I believe this to be the best currently available for acoustic surveys. It is my intention simply to draw attention to the need for further study of the statistical properties of their estimator — R. I. C. C. Francis, *Fisheries Research Centre, Box 297, Wellington, New Zealand.* (JA743)

References

Francis, R. I. C. C. 1984. An adaptive strategy for stratified random trawl surveys. N.Z. J. Mar. Freshwater Res. 18: 59–71.

Jolly, G. M., and I. Hampton. 1990. A stratified random transect design for acoustic surveys of fish stocks. Can. J. Fish. Aquat. Sci. 47: 1282–1291.

Reply to Comment by R. I. C. C. Francis

We appreciate this constructive comment and its supportive references both to our paper in general and specifically to our section on model-based estimation.

The main criticism is that we did not address the question of possible bias resulting from the two-phase sampling procedure. We accept and agree that this was an omission. However, we have recently been investigating the problem and hope to publish a note in due course. Meanwhile, a few remarks may be helpful.

In general there are two ways of approaching suspected bias. One is to amend the sampling procedure so as to avoid bias; the second is to devise an appropriate correction with associated variance for the corrected estimate. S. K. Thompson (Department of Mathematics and Statistics, Auckland University, Private Bag, Auckland, New Zealand), F L. Ramsey (Department of Statistics, Oregon State University, Corvallis, OR 97331, USA), and G. A. F. Seber (Department of Mathematics and Statistics, Auckland University, Private Bag, Auckland, New Zealand) (in prep.) exemplify the first option in their description of a method of a succession of "primary units or strata" in which sampling intensity can be progressively increased as density increases. This has been applied in trawling for shrimp which form distinct but unpredictable aggregations, and the sampling intensity for a stratum is based on the shrimp density encountered in the previous stratum. Since dependency is between adjacent strata rather than within strata, no bias results.

Other adaptive techniques may be found for specific situations. An advantage of the two-phase procedure of Jolly and Hampton (1990) is its wide applicability. However, Francis (1984), in a two-phase method somewhat similar to our own where sampling intensity in the second phase depends on the stratum variances (or means) in the first phase, has demonstrated the existence of bias using simulations. In comparing one-phase with two-phase simulated surveys of mackerel and roughy, he obtains respectively mean biases of -7.2 and -3.3% for the two-phase case.

Our recent investigations (in prep.) confirm that when allocation is based on the observed variances from phase 1 (our equation (9), p. 1286) the two-phase approach produces a small negative bias which is a function of the covariance between each first-phase stratum mean and its standard deviation. Evaluation of the function involves a regression to express standard deviation in terms of mean density. To obtain a precise estimate of this regression would usually require more data than from a single survey. Nevertheless, as an illustration, we can quote the correction so estimated from the four strata in the 1985 anchovy survey of Jolly and Hampton (1990). Using the values in their table 3 together with a breakdown of numbers of transects, n_i, into first and second phase, the bias in mean density is estimated as -0.35 tonnes/km^2. This gives a corrected mean density of $9.20 + 0.35 = 9.55$, hence a bias of -3.7% of the corrected value. A similar calculation for the 1986 survey in which there were six strata gave a bias of -1.9%; the two-phase procedure in this survey was applied only to the two strata accounting for the major part of the anchovy population.

Although it appears that the allocation procedure of Francis (1984) is equivalent to ours, we are uncertain why he adopts such a relatively complex procedure. This may or may not be concerned with the practicalities of the decision making in relation to the ship or ships involved, location of strata, etc., but this is not discussed. However, we dispute the suggestion that our allocation procedure leads to difficulties. Either his or our method can lead to "oversampling" of particular strata in phase 1 if the initial information on variances is inaccurate. This leads to a "negative" allocation in phase 2. If a stratum has been "oversampled" in this way, we would take no phase 2 samples from that stratum and allocation for the remaining strata would be in the same proportion as originally intended. No information, of course, would be discarded from the "oversampled" stratum. We had thought that this strategy would be apparent given that the overriding constraint was total sailing time available (p. 1286, para. 4).

We do not believe that our policy of rounding allocations to the nearest integral numbers could have any perceptible effect on the final variance even if this did occasionally mean that, in integral terms, we had departed slightly from the theoretical optimum; the final variance is certain to be very insensitive around its minimum value to such rounding-off errors. Also, although the alternative procedures appear to be otherwise identical, Francis (1984) gives no proof that his, like ours, is based on the classical Neyman allocation (see Cochran 1977, chap. 5).

In the final paragraph of our paper we are merely visualising circumstances where the two-phase approach might have disadvantages, namely when there is substantial fish movement. In such a situation, bias, increased random variation, or both could occur, depending on the interrelationship between movement and the survey design. Francis (1984) also refers to possible bias from this source (p. 59). Since, with movement normal to the transects, the effect would be maximum, the effect could be increased still further by the time gap between phases 1 and 2 (as mentioned three paragraphs earlier in our paper). Thus, in the context of the anchovy survey where the highest densities occur in the west, and irrespective of the direction of movement, the time gap could be most effectively reduced for the majority of the population by conducting phase 1 of the survey in an east to west direction — G. M. Jolly, *38 Silverknowes Terrace, Edinburgh, EH4 5HJ United Kingdom,* and I. Hampton, *Sea Fisheries Research Institute, Private Bag X2, Rogge Bay, Cape Town, Republic of South Africa.* (JA826)

References

COCHRAN, W. G. 1977. Sampling techniques. 3rd ed. John Wiley and Sons, New York, NY. 428 p.

FRANCIS, R. I. C. C. 1984. An adaptive strategy for stratified random trawl surveys. N.Z. J. Mar. Freshwater Res. 18: 59–71.

JOLLY, G. M., AND I. HAMPTON. 1990. A stratified random transect design for acoustic surveys of fish stocks. Can. J. Fish. Aquat. Sci. 47: 1282–1291.

ANNOUNCEMENTS/AVIS

Aquatic Toxicity Workshop

The 18th Annual Aquatic Toxicity Workshop will be held in Ottawa, Ontario, from 30 September to the 3 October 1991. The theme of the workshop will be Ecological Perspectives on Aquatic Toxicology. Major topics will include (1) contaminant transfer through the abiotic and biotic compartments of the environment, (2) population and community responses to stresses, and (3) the role of aquatic toxicology in the regulatory agenda. A plenary session will be held on "Perspectives on aquatic toxicology: the government and research communities."

A special Arctic session "Contaminants in Arctic Ecosystems" will include speakers from Sweden, Norway, Denmark, USSR, the United States, and Canada.

Platform and poster sessions will be accepted in English or French (there will be simultaneous translation) and abstracts must be received by 2 July 1991 to be considered. For further information: Dr. Margaret C. Taylor, Water Quality Branch, Environment Canada, Ottawa, Ontario K1A 0H3, Canada. Telephone (819) 953-1553; FAX (819) 997-8701.

Atelier sur la toxicité aquatique

Le dix-huitième atelier annuel sur la toxicité aquatique se tiendra à Ottawa (Ontario) du 30 septembre au 3 octobre 1991 et aura pour thème les perspectives écologiques sur la toxicité aquatique. Parmi les principaux sujets qui y seront abordés, citons : 1) le transfert des contaminants par les composantes biotiques et abiotiques de l'environnement, 2) les réponses des populations et des communautés aux stress et 3) le rôle de la toxicologie aquatique dans le plan d'action sur la réglementation. Une séance plénière concernant les vues des fonctionnaires et des chercheurs sur la toxicologie aquatique sera aussi au programme.

Au cours d'une séance spéciale portant sur l'Arctique et intitulée « Les contaminants dans les écosystèmes arctiques », on pourra entendre des conférenciers en provenance de la Suède, de la Norvège, du Danemark, de l'URSS, des États-Unis et du Canada.

La Direction acceptera jusqu'au 2 juillet 1991 les résumés des communications en anglais ou en français pour les séances thématiques et celles d'affichage (un service d'interprétation simultanée sera disponible). Pour obtenir de plus amples renseignements à ce sujet, prière de communiquer avec Mme Margaret Taylor, Direction de la qualité des eaux, Environnement Canada, Ottawa (Ontario) K1A 0H3, Canada. Téléphone (819) 953-1553; télécopieur (819) 997-8701.

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